OF MICE AND HOMININS:

USING THE CRANIOMANDIBULAR MORPHOLOGY OF HYBRID MICE TO BETTER UNDERSTAND HYBRID MORPHOLOGIES IN THE HOMININ FOSSIL RECORD

by

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DECLARATION

I, Kerryn Ashleigh Warren, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

This thesis has been submitted to the Turnitin module, and I confirm that my supervisor has seen my report and any concerns revealed by such have been resolved with my supervisor.

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ABSTRACT

Since the sequencing of the Neanderthal genome in 2010, there has been an explosion of molecular research into hybridization and gene flow among hominin taxa in the Late Pleistocene. However, little research has focussed on how hybridization affects skeletal morphology. In regions and time periods where the recovery of ancient DNA is not possible, a thorough understanding of hybrid morphologies is essential for truly understanding hominin interactions in the past. This thesis examines the cranio-mandibular morphologies of hybrid mice across different degrees of phylogenetic relatedness (three sub-specific hybrids and one specific hybrid) and through several generations (F1s, B1s and F2s for the sub-specific hybrids), in order to build an animal model for better understanding hybrid morphologies. Cranio-mandibular size, form and shape are compared between parents and hybrids (N=634), as are frequencies of unusual non-metric traits. Morphometric analyses show that all first generation (F1) hybrids are intermediate in cranial and mandibular shape, and larger in size than the mid-parental mean, or sometimes even larger than parents. However, the expression of these differences in hybrids appears to be dependent on phylogenetic distances between parents, with sub-specific F1 hybrids often appearing transgressive (outside the range of both parents), and specific hybrids more intermediate. Subsequent hybrid generations (B1s and F2s) are highly variable in cranio-mandibular size and shape depending on the generation of the cross, possibly reflecting the degree of heterozygosity. B1s and F2s are highly variable, with examples of both parental morphologies as well as hybrid heterotic size being retained in some individuals. Models based on these data show that it is possible to detect hybridization in samples (as opposed to sampling sympatric non-hybridizing taxa) on the basis of morphological variability. In terms of non-metric cranial traits, hybrids are more likely to express unusual sutural anomalies and atypical bilateral foramina. Two specimens (intra-specific F1 and B1 individuals) showed extensive wormion bones. These data corroborate current research on hybrids, providing further evidence for the patterns seen in other animal hybrids. Furthermore, results of this study support morphological evidence for hybridization in several hominin specimens, including Oase II (cranium associated with a known multigenerational recombinant) and potentially other Middle Pleistocene hominins.

To the women that have inspired and encouraged me, and the men that have made our journeys easier.

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We will now discuss in a little more detail the struggle for existence —

Charles Darwin (On the Origin of Species)

CHAPTER 1

INTRODUCTION

In 1980, Jean M. Auel published her epic novel, *Clan of the Cave Bear*. The story follows a young human girl who is separated from her family after a natural disaster, and is fostered by a group of Neanderthals. She eventually falls pregnant by one of the young adults and gives birth to a son. While the story itself is fictional, it was inspired by many of the paleoanthropological debates of the time, particularly around the relative humanity of Neanderthals. Could they speak like us? Did they think like us? Could they breed with us? And, most importantly for this thesis, when they bred with us, what did the offspring look like? In the story, the hybrid child appeared deformed and infantile to the Neanderthals: more human-like.

Thirty years later, the first high-coverage sequencing of the Neanderthal genome revealed that humans did interbreed with Neanderthals, after leaving Africa between 100-50 Ka (thousand years ago; Green, Krause *et al.* 2010). Peoples with recent origins outside of sub-Saharan Africa have been shown to possess between 1-4% of the Neanderthal genome. Furthermore, subsequent research has shown that modern humans all over the world show variable evidence for past hybridization

between us and multiple, some yet unknown, species of hominins, both within Africa and outside of Africa (Reich, Green *et al.* 2010, Hammer, Woerner *et al.* 2011, Kuhlwilm, Gronau *et al.* 2016). Hybridization events in the Late Pleistocene were rife.

While we can safely say that hominins, including humans and Neanderthals, hybridized in the past, we still have many unanswered questions. When and where did hybridization take place? Were the populations merged socially as well as biologically? What did these hybrids look like, morphologically? And was any morphological "mixing", or other change that may occur in the hybrid morphology, transferrable to subsequent multigenerational recombinants? Moreover, while advancement in ancient DNA technologies has been incredible, they are likely not sufficient for analysing the bulk of the human fossil record, including earlier hominins, and hominins found in sites which are unfavourable to DNA preservation. Clearly we need to better understand the link between what we know about hominin hybridization from the genetics, and what we know about hybrid morphologies from hybrids of living species, in order to better understand the paleoanthropological record and the role of hybridization in human evolution.

Multiple mammalian species hybridize, including many primate species (Arnold, Meyer 2006). We know a little about the effects of hybridization on the cranium of baboons, gorillas, tamarins and other mammals, but the majority of these studies have been conducted on curated collections (such as museum samples), where the genealogies of the specimens are unknown or shallow (Ackermann, Schroeder *et al.* 2014, Ackermann, Brink *et al.* 2010, Ackermann, Rogers *et al.* 2006, Ackermann, Bishop 2010, Fuzessy, de Oliveira Silva *et al.* 2014, Ackermann 2009, Eichel, Ackermann 2016, Cheverud, Jacobs *et al.* 1993). Large, controlled databases of mammalian hybrids are necessary in order to understand the patterns of these hybrid morphological effects. The Hybrid Mouse Project (PI: Rebecca Ackermann), aims to do just that. This study focusses on one aspect of the larger project, by studying the morphology of the crania and mandibles of mice and their hybrids.

THIS STUDY

In this study mice are used as a model organism to explore the cranio-mandibular morphology of hybrids. Two species of *Mus* and three subspecies of *Mus musculus* are used in this thesis to assess the similarities and differences in the morphological patterns produced from the hybridization between parent taxa (among subspecies and species), F1 (first generational) hybrids, and

multigenerational recombinants (hybrids crossed together, or F2s; and hybrids crossed with parent taxa, or B1s). Mice are ideal model organisms for understanding evolutionary outcomes. They have short life-spans, are relatively quick to breed and have been extensively researched. Comparing the morphology seen here with what has been researched on hybrids of other mammals, including primates, and wild taxa, may allow us to create a model for understanding and predicting mammalian hybrid morphologies.

This thesis is divided into nine chapters. **CHAPTER 2** expands on the evolutionary consequences of hybridization on numerous animal taxa (mammalian in particular). This chapter also unpacks current hypotheses on how hybridization impacts speciation and species-success, and introduces fundamental concepts on how hybridization occurs in natural populations. **CHAPTER 3** looks more specifically at hybrid morphologies (skeletal, pelage and body size), as understood from the current literature. Hybrid morphologies from numerous animal species (particularly primates) are discussed, as well as the potential underlying developmental mechanisms which bring about these morphologies. These chapters are important for laying the theoretical and biological groundwork for understanding the role of hybridization in human evolution, which shall be more directly explored in **CHAPTER 4**. Here the recent history shaping our understanding of modern human origins, and hybridization among hominin taxa, is explored. This chapter also discusses how recent successes in ancient DNA retrieval and analyses have helped shape our current understanding of hybridization, both in the Late Pleistocene and among earlier hominins. This chapter also looks back at debates in the past which focussed on hybridization among modern humans and Neanderthals, since these debates focussed largely on the morphology of individual specimens. In hindsight, this information may provide further clues as to how to detect hybridization in hominins in the future, and what went wrong (and right) in the debates of the past.

CHAPTER 5 is the Materials and Methods chapter. It introduces the mice used in this thesis, and explains how these mice, in particular, are important for better understanding the effects of hybridization. The methodology section is then divided into three sections, representing each of the subsequent results chapters. **CHAPTER 6** is the first results chapter, and a manuscript currently in review at the *Journal of Human Evolution*. This chapter uses techniques employed in previous studies of baboon, gorilla and tamarin hybrids, to analyse similarities and differences in cranio-mandibular form between intra-specific parent and hybrid mouse taxa. It is an attempt to introduce the Mouse Hybrid Project by connecting it with previous research.

CHAPTER 7 is the second results chapter, exploring mouse cranial and mandibular shape and size using Geometric Morphometric techniques. This chapter also looks at multigenerational

recombinants (B1s and F2s), to see whether any size and shape changes in the F1 hybrids are retained in these subsequent generations. In this chapter, inter-specific hybrid cranial morphology is also examined, to better understand how phylogenetic distance between parents may influence the morphology of hybrids. **CHAPTER 8** is the final results chapter, and looks at cranio-mandibular non-metric traits of the strains analysed in chapter 7. Here we assess if and how hybridization effects the expression of discrete traits such as those seen on the teeth, sutures and foramina of the cranium and mandible in other primate studies (e.g. Ackermann *et al.* 2006, 2014). **CHAPTER 9** is the Discussion and Conclusion, where the background knowledge will be integrated with the results. Here we see how the morphologies detected in the hybrid mice compare with previous studies on animal hybrid morphologies, and build models to interpret these results. It is also here where an attempt is made to extrapolate our results onto the hominin fossil record, and discuss how this research can be further refined or expanded upon in the future.

SUMMARY OF HYPOTHESES

The overall objective of this thesis is to understand mammalian hybrid morphologies using a hybrid mouse model. To achieve this, each results chapter explores several hypotheses, many of which have initially been laid out in Ackermann (2010). In general, the purpose of the three results chapters (6-8) can be summarised as follows: Chapter 6 examines cranio-mandibular differences between parents and F1 hybrids in form, size and (briefly) in shape, connecting research from museum-collected baboon, gorilla and tamarin data to the intra-specific hybrids in the mouse project. Chapter 7 further explores the cranio-mandibular morphology, focussing on multigenerational recombinants, and includes inter-specific F1 hybrid cranial morphology. Here it was possible to see whether F1 hybrid morphology can be retained in subsequent generations, and the potential effects on morphological variation in mixed hybrid populations. Chapter 8 quantifies cranio-mandibular non-metric trait variation in hybrids relative to parental taxa. The hypotheses tested in this thesis can therefore be summarised as follows:

CHAPTER 6

HYPOTHESIS 1: F1 hybrids are larger than a calculated midpoint of parents in measures of cranial and mandibular form relative to the parents.

HYPOTHESIS 2: The degree of heterosis seen in F1 hybrids relative to their parent taxa is comparable with that seen in other mammalian hybrids (baboons, tamarins, etc).

HYPOTHESIS 3: F1 hybrids exhibit intermediate shape relative to their parent taxa.

CHAPTER 7

HYPOTHESIS 1: Like F1 hybrids, multigenerational recombinants are larger than parents.

HYPOTHESIS 2: We can use size variables to test for hybridization within a mixed sample. There is an increase in absolute size and size variation of a sample if hybrids are included in that sample, as opposed to a sample with only parents.

HYPOTHESIS 3: Multigenerational recombinants are intermediate in shape relative to parents.

HYPOTHESIS 4: F2 and B1 generations are more variable in shape than parents or F1 hybrids.

HYPOTHESIS 5: F2 hybrids overlap in shape with the F1 hybrids, and B1s are intermediate in shape, between hybrids and parents.

HYPOTHESIS 6: There is a breakdown in integration and covariation of the cranium and mandible in subsequent multigenerational recombinants compared with parents.

CHAPTER 8

HYPOTHESIS 1: Atypical non-metric trait variation occurs at a higher frequency in hybrids and multigenerational recombinants, relative to parents.

HYPOTHESIS 2: Atypical non-metric traits are more likely to occur bilaterally in hybrids, relative to parents.

Hybridization may be common and widespread, spatially or temporally localized or globally rare. It may influence a rare interacting population much more strongly than an abundant population, and its consequences may depend on whether populations are growing or contracting, local or invasive.

Abbott *et al.* 2013, 230

CHAPTER 2

HYBRIDIZATION IN ANIMALS: EVOLUTIONARY CONSEQUENCES

Hybridization is the "crossing of genetically distinguishable groups or taxa, leading to the production of viable hybrids" (Mallet 2005, 230; see also Barton, Hewitt 1985, Abbott *et al.* 2013). The Biological Species Concept (BSC), proposed by Mayr and Dobzhansky in the first half of the 20th century, states that species are separated once they can no longer potentially interbreed (Dobzhansky 1940, Mayr 1963). Within Mayr's model, there are several stages of speciation, but the effect of having the BSC as the predominant model governing species classification and understanding, was one of typology based on interbreeding. The extensive use of the BSC by evolutionary biologists and conservationists in the twentieth century was under the assumption that hybridization is unusual, and only occurs in circumstances such as in a controlled laboratory environment (or imagined in science fiction). Hybridization was not seen as a major contributor to evolution (but see Anderson, Stebbins Jr 1954, Anderson, Hubricht 1938, Anderson 1948).

Even when hybrids were observed in nature historically, they were often treated with disbelief. For example, when Bernstein (1966) reported to have identified two macaque hybrids, he expressed surprise that this phenomenon occurred in the wild. While the discovery largely attributed these hybrids to habitat disturbance (and therefore it was still considered "unnatural"), it did show that hybridization in the wild was possible (even if under unusual circumstances). However, anthropomorphic environmental disturbances and the associated range changes of animals, as an explanation for the occurrence of hybridization, still championed the BSC by suggesting that without these disturbances it would not happen (i.e. it still wasn't perceived as natural). Even if hybrids occurred they were typically assumed to be less viable or infertile, and rare introgression events were assumed to be deleterious (Mallet 2005). Hybridization was thought of as "the grossest blunder in sexual preference which we can conceive of an animal making" (Aylmer 1930). We now know that while this may indeed be the case in some instances, the success and results of the production of hybrid offspring are incredibly variable, as will be explored in this chapter.

While there are a number of Species Concepts from which to draw, many of which are more useful in understanding evolutionary or phylogenetic histories of organisms, or are more appropriate in understanding fossil records (such as the evolutionary species concept, which focusses on morphological differences), the BSC is still the best known and described. While this is important to understand a historic resistance to hybridization and gene flow as influential evolutionary forces, it will not be discussed further. Because this thesis focusses on the effects of hybridization, categorising taxa within species is difficult to fit within this model, particularly given the fact that living animal models (which are often categorised under a variety of species concepts, from the BSC to genetic) are being used in this thesis to understand a fossil record (where many of these concepts cannot be used). "Taxa" or "lineages" allow for more fluidity when describing divergent populations of organisms: some more closely or divergently related, some interbreeding with relative ease, some whose hybrids are more or less fit, and some which are morphologically very different from each other.

HYBRIDIZATION AS AN EVOLUTIONARY FORCE

Of the four evolutionary forces (mutation, selection, genetic drift and gene flow), only mutation and gene flow are seen as forces which contribute to, or increase, genetic variation within populations. However, only recently have hybridization and introgression (mechanisms which allow for gene

flow) been considered processes which facilitate and influence evolution (Arnold, Meyer 2006, Mallet 2005, Arnold, Martin 2009, Arnold 1992, Dittrich-Reed, Fitzpatrick 2013, Dowling, DeMarais 1993, Dowling, Secor 1997, Feder, Egan & Nosil 2012, Kronforst *et al.* 2013, Lewontin 1966, Schwenk, Brede & Streit 2008, Seehausen 2004, Stebbins 1959, Abbott *et al.* 2013, Arnold 2015). Hybridization occurs often, with approximately 10% of animal species (although admittedly rare per individual) hybridizing, or showing evidence for recent introgression (Mallet 2005, Grant, Grant 1992).

Hybridization and introgression are therefore quite common in nature, and are considered an important part of the evolutionary process (Mallet 2005, Stelkens, Seehausen 2009, Arnold, Martin 2009, Arnold 2015, Arnold 1992; but see Schumer et al. 2014). Considering the comparative rarity of mutations, hybridization and gene flow can be an even more likely or effective source of novel variation in populations: variation which could then be acted upon by natural selection (Abbott *et al.* 2013, Arnold, Martin 2009, Lewontin 1966, Arnold, Hodges 1995, Arnold 2015). In "Darwin's finches", for instance, increase in genetic variation through hybridization is between two and three orders of magnitude greater than that brought about through mutation alone (Grant, Grant 1994). Gene flow, and subsequent introgression between taxa, allow for adaptive and neutral traits to pass from one population or taxon to another, and contributing to the potential adaptability and success of the taxon. Unless there is complete allopatry or instantaneous speciation, hybridization and gene flow will continue to influence the evolution and speciation of taxa, with even low rates of hybridization impacting gene transfer (Abbott *et al.* 2013).

The role of hybridization in evolution is currently better understood, but this is still an under-explored area of research, especially in the study of human evolution. There is, however, an abundance of hybridizing taxa. Many such taxa are relatively recently divergent, but this implies that many taxa which are more greatly divergent today may have hybridized in the past, with introgression continuing long after "speciation", or initial divergence (Mallet 2005, Arnold 2015).

THE "RULES" OF HYBRIDIZATION

Despite this, hybridization events are difficult to study. While introgressive hybridization has been demonstrated in many taxa using genetic evidence, F1 hybrids are uncommon between many animal species (and therefore difficult to study directly). The rarity of first generation hybrids can be due to

prezygotic barriers (such as different habitats, breeding times, different sex organs, sexual behaviour or species-specific gametic recognition) or postzygotic barriers (such as hybrid inviability or hybrid infertility) that are likely to occur between taxa (Arnold, Martin 2009). Of course, F1 hybrids must have been produced at one point in order for these genes or traits to have introgressed from one species to another (Arnold, Martin 2009). Backcrosses between hybrid and parental species can also be difficult to identify morphologically, as they may be indistinguishable from parental populations, a phenomenon that may lead to their underestimation (Ackermann 2010, Mallet 2005).

The use of genetic markers to Understand Patterns of Hybridization

Hybridization may lead to introgression, from which, on a genomic level, multiple alleles could be selected for or against, or diffuse neutrally through the population (Arnold, Martin 2009). Furthermore, after the F1 generation, the recombination of genomes within subsequent generations are likely to be highly diverse and thus creating a large range of variation on which natural selection can act (Dittrich-Reed, Fitzpatrick 2013).

By studying genetic markers across narrow hybrid zones, hybridization and introgression among numerous taxa have been detected and characterised (Mallet 2005, Arnold, Meyer 2006). Genetic incongruence between phylogenies drawn from different genomic loci (or even phenotypic and geographic datasets) is a potential indicator of hybridization or gene flow in the past (Arnold, Meyer 2006, Linder, Rieseberg 2004). This has been used to argue for past hybridization among multiple taxa, with some sections of the genome of one taxon being more recently divergent from another taxon than any other section. This is particularly noticeable in mammals where the mitochondrial DNA (mtDNA) is more recently or greatly divergent than autosomal DNA. The mosaic nature of organismal genomes is often due to differing evolutionary histories across different parts of the genome, supporting introgression among these taxa (Arnold, Meyer 2006, Mavárez *et al.* 2006, Salzburger, Baric & Sturmbauer 2002).

Past introgression, however, is not the only explanation for genetic incongruence. Different loci indicating different evolutionary histories may also be the result of incomplete lineage sorting, or convergent evolution (Arnold, Meyer 2006, Arnold 2008, Pollard *et al.* 2006). Incomplete lineage sorting is where a polymorphic ancestral population contributes several genetic variants (for instance, alleles) to daughter populations, but these later populations differentially lose these alleles over time due to genetic drift or selection (Rogers, Gibbs 2014). This may result in populations with a mosaic genetic structure, mimicking gene flow in that some parts of the genome more closely

resemble a more divergent population. Convergent evolution, on the other hand, is often thought to be a more phenotypic phenomenon, and occurs when similar traits evolve in separate taxa due to similar selective pressures (Parker *et al.* 2013). Research has shown adaptive convergent evolution can also be detected on the molecular (protein and genomic) level as well (Parker *et al.* 2013, Liu *et al.* 2010, Castoe *et al.* 2009). Another mechanism for reticulation (network-like evolution), and genetic incongruence, is by lateral transfer (Arnold 2008, Mallet, Besansky & Hahn 2016), but this form of reticulation (and its effects) is beyond the scope of this thesis.

Despite incomplete lineage sorting and adaptive convergent evolution exhibiting similar genomic patterns of incongruence as ancestral introgression, the abundance of contemporary hybridization among related or descended taxa offers further support for hybridization in the past. For instance, hybridization among contemporary primate taxa offers some support for hybridization in the past if similar populations exhibit genetic incongruence (Arnold, Meyer 2006). Furthermore, some loci are more indicative of introgression than others: i.e. selection of introgressed loci is more effective if spread over many loci, allowing for wider clines between populations or taxa (Barton, Hewitt 1985, Mallet 2005).

Understanding patterns within the genome is therefore important for identifying past hybridization. It is also an important tool in understanding reproductive barriers among taxa, which is often thought to enhance speciation (Baird, Macholán 2012, Rieseberg, Carney 1998). Ultimately, reproductive potential depends on whether hybrids, themselves, are viable. Postzygotic isolation has been explained using a number of theories, such as Haldane's Rule of hybrid male infertility (in mammals), and by the finding of potential hybrid sterility genes between certain taxa (Mihola *et al.* 2009, Ting *et al.* 1998, Nosil, Schluter 2011). Haldane's Rule states that F1 hybrids of the heterogametic sex (in mammals this is males), are more likely to exhibit infertility (Haldane 1922). This has further implications; mitochondrial introgression is more common in mammals (including mice; Payseur, Krenz & Nachman 2004), since female hybrids are more likely to be viable.

In a similar vein, the "large X effect" hypothesizes that the X chromosome supports a large proportion of genes involved in reproduction and, thus, reproductive isolation (Masly, Presgraves 2007, Presgraves, Soojin 2009, Coyne, Orr 2004). This has been demonstrated in plants, flies and amphibians (Masly, Presgraves 2007, Hu, Filatov 2016, Dufresnes *et al.* 2016). Both Haldane's Rule and "large X effect" in Howler monkey hybrids (*Alouatta pigra* x *A. palliata*) have been supported by proportions of genetic markers across different parts of the genome (Cortés-Ortiz *et al.* 2015, Cortés-Ortiz *et al.* 2007). It is possibly also supported in past hominin introgression: by the dearth of

Neanderthal X-chromosome sequences in modern humans (Sankararaman *et al.* 2014, Vernot, Akey 2014).

Yet there are other explanations for reduced hybrid viability. Accumulations of autosomal mutations during divergence among different lineages may also play a role in enhancing speciation. Dobzhansky-Muller incompatibilities, when a new combination of alleles at two loci could be detrimental to the hybrid's survival, may also explain reduced hybrid fitness or success (Abbott *et al.* 2013). Despite the effects of selection on reproductive barriers between taxa, reproductive incompatibility generally increases with phylogenetic (and, less specifically, temporal) distance between the taxa (Abbott *et al.* 2013, Mallet 2005).

Of course, behaviour (such as assortative mating), and other factors effecting prezygotic isolation, may also prevent mating and hybridization (Mallet 2005, Abbott *et al.* 2013, Coyne, Orr 1997). It is also important to note that not all hybrids exhibit reduced fertility/viability. Some of the effects of hybridization may even be increased fitness or the formation of a hybrid taxon/lineage, but this will be more thoroughly discussed later in the chapter.

HYBRIDIZATION IN PLANTS

While zoologists and paleoanthropologists have, for much of the 20th century, ignored the potential importance of hybridization in the evolution and ecology of various taxa, botanists have not (Mallet 2005, Mallet 2007). Many plants undergo hybridization in the form of allopolyploidy (or genome doubling; Mallet 2007), where genomes are not separated by meiosis, and duplicated chromosomes are derived from separate taxa. These are particularly interesting in that many allopolyploid plants exhibit a form of hybrid vigour, which has been used extensively in agriculture (Arnold 2008, Rieseberg, Carney 1998). For example, triticale, a resilient, starchy grain, is a hybrid with four chromosome sets from wheat and two from rye: an allopolyploid (Stace 1987); the two dominant commercial species of cotton are tetraploid, with allopolyploidy possibly derived from parent lineages from both Asia/Africa (maternal) and the Americas (paternal; Arnold 2008); and different combinations of chromosomes from different progenitor lineages form the large variety of crops in the *Brassica* family (e.g., turnips, cabbage, kale, horseradish, mustards and broccoli; Lysak *et al.* 2007, Nagaharu 1935, Arias *et al.* 2014). The list of agricultural plants with suspected or confirmed allopolyploid origins is large and includes coffee, okra, peanuts, oats, quinoa, peppermint, banana, tobacco, cherries, plums and sugarcane (Schafleitner *et al.* 2013, Ansari, Thomas 1983, Heslop-

Harrison, Schwarzacher 2007, Maughan *et al.* 2004, Kenton *et al.* 1993, Harley, Brighton 1977, Tavaud *et al.* 2004, Hartmann, Neumüller 2009, D'Hont *et al.* 1996, Arnold 2008).

However, the lineages we are particularly interested in for the purposes of this thesis breed sexually, and morphological and physiological effects of hybridization (such as hybrid vigour) are not as easy to explain within a diploid hybridization/homoploid genome context. Sexually reproducing diploid plant hybrids (such as *Helianthus*-the sunflower- and *Iris*) may have the advantage of informing on the effects of such kinds of hybridization, while, in the case of plants, drawing from an extensive history and theory. The long literature on plant hybridization includes characterization of sterility and inviability in hybrids (Rieseberg, Carney 1998). For instance, asymmetric hybridization (where parentage from one taxon is skewed towards one of the sexes) has been demonstrated in *Haplopappus* hybrids, Louisiana irises and sunflowers (Rieseberg, Carney 1998). Similarly, mapping chromosomal or genetic sequences potentially involved in hybrid sterility is easier in plants (and has been demonstrated in hybrids among lentil and sunflower taxa; Rieseberg, Carney 1998).

Plants are also excellent models for hybrid vigour. While hybrids are highly variable in fertility and fitness, many exceed their parents in size or robustness: i.e. they may be transgressive to their parents (Rieseberg, Carney 1998). Transgressive phenotypes may ultimately become fixed and selected for within a population, leading to hybrid speciation. This has been shown to have profound evolutionary effects on wild sunflower hybrids, where taxa found in extreme environments, have hybrid origins (e.g. *Helianthus paradoxus,* thrives in salt marshes. This species has been shown to have hybrid origins of the taxa *H. annus* and *H. petiolaris*, which do not; Lexer *et al.* 2003, Rieseberg *et al.* 2003). Synthetic sunflower hybrids have been shown to have similar resistance to harsh environmental conditions. This, together with complementary Quantitative Trait Loci (QTLs) correlated with some transgressive phenotypes in these synthesized hybrids, supports the idea that such robustness in the hybrid taxa are due to selection of complementary gene action (although epistatic effects were also observed; Rieseberg *et al.* 2003). Hybrid speciation has also been detected in *Iris nelsonii*, a natural Louisiana Iris taxon derived from hybridization among three other Iris taxa (Randolph 1966, Arnold, Bennett & Zimmer 1990, Arnold 1993).

Experiments on Iris recombinants have also shown that adaptive introgression of alleles between parent groups adapted to wet (*I. fulva*) and dry (*I. brevicaulis*) habitats are complex (Arnold 2009). In studies subjecting recombinants to dry and flooded conditions, introgression of certain alleles reduced survivability in dry habitats and increased survivability during flooding (Martin, Bouck & Arnold 2006, Martin *et al.* 2005).

Many of the points brought up in this section (hybrid speciation, adaptive introgression) will be expanded on later in the chapter with respect to animal hybridization. It is important to realise, however, that much of the theoretical knowledge of the evolutionary effects of hybridization has been derived from studies involving plant hybrids. In these ways, and others, much of our understanding of the effects of hybridization comes from plants, but plants are imperfect models for understanding hybridization in animals for a number of reasons. Most pertinently, animal taxa are largely confined to homoploid hybridization. Animal hybridization is also affected by avoidance mating strategies, where a number of indications (smell, mating call, colour of wings, etc.) may affect mate choice, preventing them from mating with individuals from more divergent populations. Mammalian hybridization, in particular, is likely effected by limitations imposed by gestation and maternal care.

Hybridization in Animals

Hybridization has also been influential in the evolutionary history of animals. While not as common as in plants, allopollyploidy does play a role in the reticulate history of unisexual insects and lizards (Bullini, Nascetti 1990, Sites Jr *et al.* 1990, Vrijenhoek 1989). Similarly, polyploidization, resulting in two genome duplications, occurred early in the evolution of vertebrates (half a billion years ago; Dehal, Boore 2005). While it is unknown as to whether this was the result of spontaneous duplication or allopoyploidy, this had a profound impact on the evolution and complexity of vertebrates (Lynch, Walsh 2007). Thus, while allopolyploidy is common among plant hybrids, animal hybrids generally maintain a diploid genome structure—homoploid hybridization— (Mallet 2007, Arnold 2008), from which backcrossing, introgression and hybrid speciation may occur. This kind of hybridization is more relevant to the topics discussed in this thesis.

Homoploid hybridization occurs in many animal taxa. In the wild, homoploid hybridization and introgression among numerous insect taxa has been reported, including fruit flies (Coyne, Orr 1997, Yukilevich 2012, Nosil 2013), butterflies (Kunte *et al.* 2011, Mallet, McMillan & Jiggins 1998, Mavárez *et al.* 2006, Cianchi *et al.* 2003, Beltran *et al.* 2007, Gompert *et al.* 2006), grasshoppers (Vedenina *et al.* 2013, Bridle, Baird & Butlin 2001, Butlin, Hewitt 1985, Arnold, Shaw & Contreras 1987, Daly, Wilkinson & Shaw 1981, Ferris *et al.* 1993), mosquitos (Turner, Hahn & Nuzhdin 2005), ground beetles (Garnier *et al.* 2006) and ticks (Rees, Dioli & Kirkendall 2003, Sutherst 1987). What is particularly informative is that the rates of hybridization in various insect groups differ substantially: even among butterflies *Heliconius* has a greater proportion of taxa which hybridize (24%) than do European butterflies (12%; Mallet, McMillan & Jiggins 1998). However, most hybridizing taxa of

Heliconius have less than 2% mtDNA sequence divergence, and no hybrids are found among species with more than 10% mitochondrial DNA (mtDNA) differences (Mallet 2005).

Hybridization is also common in fish, amphibians and reptiles (Scribner, Page & Bartron 2000, Hubbs 1955). Multiple fish and aquatic animals display mosaic or recombinant genomes, with a range of phenotypic and genetic outcomes (Avise 2000, Arnold 2009). For instance, introgression has made a profound effect on the genetic diversity of trout, with evidence for introgression in 18% of brown trout (*Salmo trutta*) populations sampled in the Iberian Peninsula (Presa *et al.* 2002). Similarly, adaptive radiation in African cichlids is proposed as resulting from past hybridization acting as a catalyst for diversification within the taxon, and the formation of hybrid species (Seehausen 2004). Non-congruency between morphological and mtDNA phylogenies has shown ancient and recent introgression among taxa of peacock bass, or South American cichlids (Willis *et al.* 2007). In the transition zone between the Baltic (low salinity) and North Seas (high salinity), significant introgression was noted for marine fishes such as turbot, where little gene flow is detected outside of the transition zone (Nielsen *et al.* 2004). Aquatic animals also provide examples for the formation of hybrid swarms (among subspecies of blue-gill sunfish), unidirectional hybridization (in treefrogs in Alabama), and introgressive swamping (of spotted bass into the smallmouth bass population of lake Chatuge; Avise 2000). Hybrids between native Californian Tiger Salamanders and introduced Barred Tiger Salamanders express hybrid vigour, and the hybrid population has ultimately replaced the native population (Fitzpatrick, Shaffer 2007). Hybridization has also been shown to occur between species of gartersnake, although parental traits are under strong selection (Fitzpatrick, Placyk *et al.* 2008).

Similarly, widespread introgression among bird taxa provides further evidence for the abundance and evolutionary potential of hybridization in complex, sexually producing organisms (Grant, Grant 1992, Grant, Grant 1996, Grant, Grant 1994, Elgvin *et al.* 2011, Mallet 2005). Nine percent of bird taxa are able to hybridize with another species, 19.5% of which are intergeneric (Mallet 2005). This is highly variable among different orders of birds: among Birds of Paradise (Paradisaeidae), 42.9% of species are able to hybridize with at least one other taxon; among British ducks (Anatinae), 76.2%; British Native grouse (Tetraonidae), 100%; American warblers (Parulidae), 24.1%; World tits (Paridae), 28.6%; and among western Paearctic warblers (Sylviidae), 0% confirmed, although at least one is suspected (Mallet 2005, Grant, Grant 1992, Parmenter, Byers 1991, Millais 1894, Gillham, Gillham 1996, Harrap, Quinn 1996, Arnold 2009). On a per-individual rate, however, hybridization occurs in less than 0.1% of birds (Mallet 2005).

Among animals, mammals are argued to evolve hybrid inviability much faster than birds and reptiles (Prager, Wilson 1975, Fitzpatrick 2004, Wilson, Maxson & Sarich 1974). This may be due to faster developmental change and regulatory evolution leading to accumulation of incompatibilities among mammalian taxa (Prager, Wilson 1975, Fitzpatrick 2004). It has also been suggested that greater divergence between mammalian mother and foetus may result in negative immunological reactions, which is not a problem for egg-laying animals (Wilson, Maxson & Sarich 1974). It is also possible that mammals more easily identify hybrids and members of another species and avoid interacting or mating with them. Such avoidance behaviour may further limit gene flow back into parent lineages; this may explain why only 6% of European mammalian species hybridize (Mallet 2005). The lower average rate of hybridization in European mammals compared with other animal species, such as birds, may also reflect a lack of species diversity in European mammals (Mallet 2005). In areas with greater numbers of mammalian species, there will probably be greater proportions of hybridizing mammals. It is therefore possible that mammalian hybridization occurs at a similar rate to birds, despite the above potential limitations.

Popular examples of hybrid mammals include the mule and the liger. The mule is the hybrid offspring of a male donkey and female horse (a hinny is the reverse cross), two taxa that have different numbers of chromosomes (Eldridge, Blazak 1976). The hybrid vigour displayed in the mule (in both cognition and in size; Proops, Burden & Osthaus 2009) makes them attractive working animals for farmers. Morphologically, however, they are variable, often displaying a mosaic of parental traits. Mules are renowned for displaying reduced fertility and increased sterility relative to the parent taxa (Zong, Fan 1989). The liger, a cross between a male lion and female tiger (a tigon is the reverse cross), is also well known for displaying transgression: ligers are larger than either parent taxon. Although rare, ligers do exist in captivity, and backcrosses between female ligers and parents have been recorded. Contrary to ligers, tigons do not necessarily exceed the size of the parental taxa.

Although the above are well-known examples of mammalian hybrids, hybridization occurs naturally and successfully across multiple mammalian taxa, and has shaped the evolution of many more. Indeed, hybridization in cats is not just limited to lions and tigers, but has been seen among multiple felid taxa, domestic and in the wild (Trigo *et al.* 2013, O'Brien, Koepfli 2013, Trigo *et al.* 2014, Nussberger *et al.* 2014). Hybridization also occurs amongst dolphins (Amaral *et al.* 2014), whales (Heide-Jørgensen, Reeves 1993), genets (Gaubert *et al.* 2005), marmosets (Malukiewicz 2013, Malukiewicz *et al.* 2014, Fuzessy *et al.* 2014), squirrels (Chavez, Saltzberg & Kenagy 2011, Thompson *et al.* 2013, Goodwin 1998), woodrats (Coyner, Murphy & Matocq 2015), wildebeest (Brink 2005,

Ackermann *et al.* 2010), deer (Stubblefield, Warren & Murphy 1986), chipmunks (Good *et al.* 2008), rabbits and hares (Matthee *et al.* 2004, Zachos *et al.* 2010), and mice taxa (Pallares, Turner & Tautz 2016, Mikula, Auffray & Macholan 2010, Hauffe, Giménez & Searle 2012, Alibert *et al.* 1997, Baird, Macholán 2012).

One mammalian group which has been extensively researched for better understanding of the effects of hybridization, and the resultant introgression are canids (particularly wolves, dogs and coyotes). Coyotes, wolves and dogs often interbreed, with limited or no reproductive isolation that allows for extensive introgression. Hybridization has also occurred between gray wolves and eastern wolves (a species, although this designation is controversial, with some believing eastern wolves to be populations originating from gray-wolf/coyote hybridization in the past; Fain, Straughan & Taylor 2010). In gray wolves (*Canis lupus*) in North America, it has been shown that the allele that allows for black colouring in wolves likely derives from various dog breeds, with possible adaptive benefits in wolves that are heterozygotic at that locus (Anderson *et al.* 2009, Coulson *et al.* 2011, Hedrick 2013). Wolf-dog hybridization also occurs in Eurasia (mostly eastern and southern Europe and the Middle East), albeit more rarely, no doubt due to wolf populations being reduced in size, fragmentary and in close proximity to humans and dogs (Andersone, Lucchini & Ozoliņš 2002, Randi, Lucchini 2002, Khosravi, Rezaei & Kaboli 2013).

Anthropogenic activities which have led to the reduction of wolf populations in North America, likely facilitated the range expansion of coyotes, which are more greatly colonising eastern North America. Part of their success in expansion may be due to introgression with wolves (which may allow for adaptation to geographic habitat and dietary niche) and dogs (which may allow for adaptation to human-dominated habitats; Monzón, Kays & Dykhuizen 2014, Kays, Curtis & Kirchman 2010). Eastern coyotes have introgressed grey wolf genes, absent from western coyotes, and populations with more introgressed wolf haplotypes exhibit larger average skull size, possibly better for hunting larger prey (Kays, Curtis & Kirchman 2010).

Thus large hybrid zones exist throughout eastern North America: between eastern wolves (*Canis lycaon*) and coyotes (Kyle *et al.* 2006, Wilson *et al.* 2009); red wolves and coyotes (Bohling, Waits 2015); between coyotes and gray wolves (Lehman *et al.* 1991); and between gray wolves, eastern wolves and coyotes (Benson, Patterson & Wheeldon 2012, Wilson *et al.* 2009). Furthermore, asymmetric sex-biased introgression of dog (*Canis familiaris*) and wolf haplotypes into eastern coyote populations has shown that male wolves and dogs initially mated with female coyotes (Wheeldon *et al.* 2013, Monzón, Kays & Dykhuizen 2014). Similarly, coyotes (*Canus latrans*) from populations which have expanded their ranges into eastern Canada have been observed with white

coats, exhibiting a recessive allele at the *Mc1r* locus, which is found in Labradors and golden retrievers (Brockerville *et al.* 2013). This indicates a high level of introgression, albeit asymmetric, which produces a wider range of variation in the new, introgressed populations.

Ancient hybridization in mammals has also been studied, thanks to better and more extensive genomic data. Among caribou/reindeer subspecies (*Rangifer tarandus*) in North America, ancient introgression may have been adaptive, with behavioural adaptations that allowed the mixed population to migrate between tundra and boreal habitats in the Canadian Rockies (Shurtliff 2013, McDevitt *et al.* 2009). MtDNA haplotypes of mountain hare found in other hare species in the Iberian Peninsula, where the former is now extinct, supports ancient introgression, although it is likely this is an example of neutral, not necessarily adaptive, introgression (Shurtliff 2013, Melo-Fereira *et al.* 2005, Alves *et al.* 2008). Ancient introgression and reticulation has been argued or demonstrated for the origins extant elephants and woolly mammoths (Arnold 2006, Arnold 2008), domesticated cattle and wild aurochs soon after domestication of the former (Götherström *et al.* 2005), African oryx (Masembe *et al.* 2005) and many others (see Arnold 2006, 2008, and Shurtliffe 2003).

Ancient introgression sometimes appears to be related to changing palaeo-climatic conditions. The wisent (European bison) is the result of hybridization between the wild ancestors of domestic cattle, and extinct steppe bison earlier than 120 Ka (Soubrier *et al.* 2016). There is evidence for alternating environmental fitness and domination in Europe between the wisent and steppe bison with environmental change over the early to middle Pleistocene (Soubrier *et al.* 2016). Similarly, polar bears and brown bears appear historically affected by climate change. Despite having diverged between 479-343 Ka (Liu *et al.* 2014), genomic discordance reveals significant sex-biased admixture in the past (Edwards *et al.* 2011, Cahill *et al.* 2013, Cahill *et al.* 2015). This has particularly been demonstrated by ancient genome analyses of bears living on islands in southeast of Alaska, dated to the warming period of the Last Glacial Maximum in the Late Pleistocene (Cahill *et al.* 2013, Cahill *et al.* 2015), when migration likely resulted from migration of brown bears in expanding ecological territories.

Gene flow has also been detected among numerous primate taxa, with over 10% of recognised primate species currently hybridizing naturally (Zinner, Arnold & Roos 2011, Arnold, Meyer 2006). This is despite a lack of genetic data among multiple Strepsirrhine, tarsiers and New World Monkey species, which could greatly increase this proportion (Zinner, Arnold & Roos 2011). These hybridization events occur on many levels, both inter-and intra- specifically among groups in the wild. It also occurs in geographically overlapping zones; and even among genera of Old World

Monkeys (Markarjan, Isakov & Kondakov 1974, Moore *et al.* 1999). Based on paraphyletic phylogenies, determined by incongruences between genetic sequences, we can assume introgression occurred multiple times in the past among the following taxa: among lemur species and subspecies (Pastorini, Thalmann & Martin 2003, Pastorini *et al.* 2009); among new world monkeys (spider monkeys, howler monkeys, marmosets and tamarins), prosimians, langurs and cercopithecines (mangabeys, baboons and macaques), among lessor apes and, even, great apes (Arnold, Meyer 2006, Ackermann, Bishop 2010, Ackermann 2010). Although such incongruences can also be explained by incomplete lineage sorting, hybridization is likely considering it occurs so frequently among living taxa today. While most evidence for hybridization in primates is genomic, some researchers have noted wide ranges of morphological variation among primate hybrids (Arnold 2008, Ackermann, Bishop 2010). Furthermore, genetic and morphological evidence of extant species has shown hybridization has occurred among multiple taxa in the past, contributing significantly to their genetic and phenotypic variation (Ackermann, Bishop 2010, Zinner *et al.* 2009). Some living primate taxa are even argued to be the result of ancient introgression (Ackermann, Bishop 2010, Ackermann 2010, Chakraborty *et al.* 2007, Osterholz, Walter & Roos 2008, Tosi, Morales & Melnick 2000, Zinner, Arnold & Roos 2009, Zinner *et al.* 2009).

HYBRID ZONES

Hybrid zones are "areas where genetically distinct groups of individuals meet, mate and leave at least some offspring of mixed ancestry" (Barton, Hewitt 1985, 335; see also Baird, Macholán 2012, Bigelow 1965). Such zones may form following secondary contact of two taxa, or contact may be continuous, but with divergent selection (Abbott *et al.* 2013). Examples of hybrid zones have already been alluded to within this chapter (e.g. marine fish in the transition zone between the North and Baltic seas). While more specific definitions of the term "hybrid zone" are varied, for the purposes of this thesis I will follow Barton and Hewitt's (1985, 115) definition that refers to a hybrid zone as a cline: "a gradient or set of gradients in morphology or gene frequency, at one or more loci". This definition is relatively broad, and it is possible that different kinds of hybrid zones may be more easily detectable via the phenotype than others.

The maintaining of hybrid zones can be summarized into three general hypotheses, governed by dispersal and selection processes. These hypotheses greatly depend on the level of fitness between the hybrids and parents:

1. The adaptive speciation hypothesis: natural selection against hybrids varies; thereby the level of fertility/reproduction between the parental taxa will vary (Arnold 1992). Natural selection has a role in increasing biological speciation or decreasing differences between taxa.

2. Bounded hybrid superiority hypothesis: hybrids are better suited to the transition environment of the hybrid zone than either parent (Arnold 1992, Barton, Hewitt 1985, Moore 1977). Both 1 and 2 could be considered dispersal-independent clines, where dispersal- or movement of the entire zone- plays a negligible role in the maintenance of the hybrid zone (Barton, Hewitt 1985).

3. Dynamic equilibrium hypothesis, or dispersal/selection balance: A balance between hybrid inferiority and parental dispersal maintains the hybrid zone, and is the most appropriate form of tension zone (Arnold 1992, Barton, Hewitt 1985).

Hybrid zones may be measured in a couple of ways. Clinal theory, for instance, measures clinal variation (slope and width of incongruity of certain features/sequences within the zone) to determine dispersal and natural selection. For instance, in the house mouse hybrid zone in Europe (*Mus musculus musculus* x *M. m. domesticus*), hybrids have reduced fertility and viability, creating a steep tension zone (30-40km) with selection against hybrids in many loci (Dod *et al.* 1993, Teeter *et al.* 2008, Teeter *et al.* 2010, Macholán, Kryštufek & Vohralík 2003, Duvaux *et al.* 2011, Boursot *et al.* 1993). Similarly cytonuclear disequilibrium/incongruence (comparing nuclear and cytoplasmic— mitochondrial or chloroplastic— DNA to test for natural selection, migration, etc.) also helps model and inform about hybrid zones (Barton, Hewitt 1985, Arnold 1992). In the European house mouse hybrid zone, markers for mtDNA and sex chromosomes indicate a far narrower tension zone than autosomal markers (Macholán *et al.* 2008, Tucker *et al.* 1992, Payseur, Krenz & Nachman 2004).

Similarly, understanding adaptation between parent groups and hybrids to different environments (by studying habitats in which they are found and the behaviours of these taxa) has also been used to model or measure hybrid zones for many organisms. These include grasshoppers (a bioclimate model for grasshopper species) and crickets (comparing soil types; Arnold 1992, Arnold, Shaw & Contreras 1987, Bridle, Baird & Butlin 2001).

One consideration with regards to hybrid zones is that they may not be fixed. The movements of clines/zones vary depending on the selection/dispersal balance of alleles and the combination of this effect at clines where different loci may overlap (Barton, Hewitt 1985, Abbott *et al.* 2013). In the absence of dispersal, a “wave of advance” of advantageous alleles may occur at a constant speed, or

the neutral drift of other alleles will maintain the original position of the cline (Barton, Hewitt 1985, 117). Of course, this could vary even at a genetic level. Even with dispersal taken into consideration, the fitness of individuals and individual alleles, the population density and dispersal rate, and the effect of gene frequencies on the population density and dispersal would all affect the movement of the hybrid zone (Barton, Hewitt 1985).

"Wave of advance" is exemplified in *Mus* specific hybrids, where adaptive introgression of a single locus greatly benefits parent taxa, despite high levels of infertility and inviability in the hybrids. The Algerian mouse, *Mus spretus* and the western house mouse, *M. musculus (domesticus*), are currently sympatric in North Africa and southern Europe. Researchers showed that warfarin (pesticide) resistance in *M. m. domesticus* is due to adaptive introgression of the vkorc1^{spr} allele (vitamin K epoxide reductase subcomponent 1) from *M. spretus* (Song *et al.* 2011). This is despite these species being strongly reproductively isolated, with vast behavioural and ecological differences, and with hybrids having far greater infertility in all males and in some females (Dejager, Libert & Montagutelli 2009). In areas where warfarin is extensively used, even less-fertile F1 hybrids have a fitness advantage (Pelz *et al.* 2005). Furthermore, *M. spretus* variants of the allele were detected (with selective advantage) in house mice in Germany, far from where the taxa overlap (Song *et al.* 2011).

The majority of hybrid zones studied are "tension zones" (number 3, above; Baird, Macholán 2012, Barton, Hewitt 1985, Barton, Hewitt 1989). Tension zones involve both selection and dispersal forces and would move in order to minimise distance with greater selection (Barton, Hewitt 1985). Similarly, these clines may, themselves be maintained by selection against hybrids in order to balance the rate of dispersal within the zone (Key 1968). Hybrid zones could be modified for a variety of reasons, often involving selection on many genes (Barton, Hewitt 1985). Hybrid zones, mainly identified through single features, frequently involve many others that range from genetic, morphological and behavioural (Barton, Hewitt 1985).

Similarly, the directionality of gene flow or hybridization will generally not be uniform, which could influence hybrid success and "shape" of the hybrid zone. Among species of tree frogs in Alabama, unidirectional hybridization, based on sex, was observed –where backcrosses to one parent always had hybrid fathers, and backcrosses to the other parent had hybrid mothers (Lamb, Avise 1986, Mecham 1960). Because of the prevalence of Haldane's Rule in mammalian hybrids, the mothers of backcrosses tend to be hybrids. This is also seen in the European house mouse tension zone, where Y chromosome introgression is limited (Vanlerberghe *et al.* 1986, Forejt *et al.* 2012).

THE EVOLUTIONARY EFFECTS OF HYBRIDIZATION

The frequency of F1 hybrid formation may be low, with fertility sometimes affected. Nonetheless backcrosses to the parent species occur and can result in the transfer of novel genetic data among the parent populations, potentially affecting the evolution of species in a number of ways: increased similarities between the two hybridizing taxa, reinforcement of reproductive barriers thus increasing speciation, increased or reduced fitness in hybrids relative to the parents, or production of distinct and successful hybrids resulting in the formation of a new "hybrid taxon". Here I shall explore these scenarios further, in order to better understand the potential importance of hybridization in the evolution of a taxon. It is important to keep in mind that any one effect, or a combination of effects, may have influenced the evolution of our own species. This will be further explored in Chapter 4.

HYBRIDIZATION MAY REDUCE VARIATION BETWEEN PARENT TAXA (AND POTENTIALLY MERGE THEM)

The most traditional view about hybridization is that it results in the merger of the two parent taxa, reducing variability between them, and potentially forming one single taxon over time (Arnold 1992). This is the most commonly understood effect of gene flow: it breaks down barriers between lineages, preventing complete speciation. It could potentially result in the reformation of a single, more diverse, taxon. In this scenario, hybridization can facilitate greater variation, but not allow for separation between parent lineages. However, it can also have adaptive outcomes for one or both parent taxa. For instance, adaptive introgression of genes and phenotypes between groups may allow for greater survival in both groups. Genes which allow for greater fitness may spread throughout both populations, and those which do not, may be lost.

It is common for taxa to exhibit mosaic genomes, where in some regions the genes have introgressed (with positive selection) from a distant interacting parent taxon (Arnold, Meyer 2006, Arnold, Martin 2009). The introgression of genes that were non-adaptive in either parent species may ultimately allow for adaptive evolution within the hybrid or introgressed population (Lewontin 1966). Sometimes this introgression, reducing variation between taxa and increasing variation within the combined population, may occur in only a few generations. This was the case in Lake Chatuge, with introgressive swamping of recently-introduced spotted bass into the local smallmouth bass population (Avise *et al.* 1997).

One of the best examples of a scenario where hybridization and introgression reduces variation between taxa (and certainly, one of the best documented examples of witnessing hybridization affecting evolutionary outcomes in nature) can be seen in Darwin's finches. Peter and Rosemary Grant are best known for their extensive documentation and research on finch species on the Galápagos Island of Daphne Major. Among some of their observations were genetic and phenotypic changes of two finch species (*Geospiza fortis* and *G. scadens*) over thirty years (Grant, Grant 2014, Grant, Grant 2002). Between 1976 and 1982, no F1 hybrids were recorded to have survived and reproduced from these taxa. Between 1982 and 1983, an El Niño event resulted in incredibly high rainfall levels, followed by several years of unpredictable rainfall fluctuations. This led to floral changes in the environment which favoured the survival of birds able to eat the abundance of small seeds. Hybrids were therefore at an adaptive advantage over one of the parent groups (*G. scadens*), which, between 1982 and 2002, became increasingly heterozygotic (Grant, Grant 1996, Grant, Grant 1992, Grant, Grant 1994, Grant, Grant 2002). This led to increasing similarity, genetically and phenotypically, between the two taxa.

Another example of increasing similarity between taxa may lie in comparisons between historic and contemporary reports on the phenotypic and behavioural differences between the mule deer (*Odocoileus hemionus*) and the white-tailed deer (*O. virginianus*) in Texas (Avey 2003). Historically, these taxa have been recorded as having significant morphological differences, and having strong preferences for different habitats (Arnold 2009). Recent analyses have not supported these reports (Cathey *et al.* 1998). This is likely due to mtDNA introgression from white-tailed into (otherwise, morphologically defined) mule deers (Cathey *et al.* 1998, Bradley *et al.* 2003). Among African elephants, *Loxodonta Africana* and *L. cyclotis*, a number of morphological and genetic features are used to differentiate between the two. However, high levels of detected admixtures, particularly of the genetic markers, have led some researchers to question the specific nature of the African elephants (Arnold 2006).

Hybridization may hasten speciation between parents

In some instances, hybridization accelerates speciation between the original taxa: reproductive barriers may be reinforced through selection, further dividing the taxa so that hybridization is increasingly rare and speciation may occur (Arnold 1992, Abbott *et al.* 2013, Dobzhansky 1940). While gene flow is often associated with reinforcing similarities between populations (and therefore a hindrance to speciation), there is increasing evidence for speciation with gene flow in a variety of plant and animal taxa (Kronforst *et al.* 2013, Mallet 2005, Mallet 2007, Coyne, Orr 1997, Ortiz-

Barrientos, Grealy & Nosil 2009). Although hybridization is most likely between more recently diverged taxa, it may persist millions of years after the initial divergence of the species (Mallet 2007), and is more likely to occur between taxa which are rapidly speciating and diversifying through adaptation. Reduced fertility, leading to biological speciation, may be selected for through reinforcement (Coyne, Orr 1997). This may occur when the hybrid is less fit than the parental species. However, this may also occur as a by-product of geographic separation or another evolutionary change (Mallet 2005).

This has been supported by research on *Drosophila* (fruit fly). *Drosophila* taxa have proven particularly informative, with numerous crossable taxa, and extensive laboratory experimentation having been conducted on them. Studies on hybridizing Drosophila have highlighted potential factors affecting genetic distance and reproductive isolation. Such studies have indicated that, while both pre- and postzygotic reproductive isolation increase with greater temporal divergence, prezygotic isolation evolves more quickly, particularly among sympatric taxa, thus showing reinforcement; and level of sympatry impacts prezygotic isolation and may play a key role in speciation (Coyne, Orr 1989a, Yukilevich 2012, Nosil 2013). Such studies on *Drosophila* have also been useful in analysing Haldane's Rule, showing that infertility and inviability typically affects the heterogametic sex (males in Drosophila) long before postzygotic isolation affects hybrids of both sexes (Coyne, Orr 1989b, Coyne, Orr 1997).

Barriers to hybridization, including environmental factors, a "hybrid sink" effect, and the effects of the combination and associations between alleles, may impede gene flow (although not necessarily select against it; Barton, Hewitt 1985). Barriers may also include Dobzhansky-Muller incompatibilities, assortative mating and ecological divergence between taxa, which may all be driven by natural selection and adaptation, as discussed earlier in this chapter (Abbott *et al.* 2013). These barriers may be asymmetric, with gene flow more favourable in one direction, owing to differences in fitness or population density or the movement of the hybrid zone. This often results in more gene flow into the less fit taxon (Barton, Hewitt 1985).

How hybridization may accelerate speciation has been modelled in several ways. One model emphasizes genomic coupling, which works as follows: genetic and morphological markers are transferred in either a quantitative (frequency of markers based on frequency and success of introgression) or qualitative (selective transfer of specific markers) manner (Arnold 1992). This leads to varying levels and combinations of markers in the introgressed populations, particularly when this process is ongoing. The strength of the association between loci which reduce potential recombination (genetic barrier loci) depends on the level of recombination and selection that

maintains these loci (Abbott *et al.* 2013). This strength of association is known as coupling, with high coupling often further separating the two parent taxa, and low coupling favouring recombination since the barrier loci are acting independently of each other, and are thus ineffective at preventing biological interaction. Coupling may play an important role in hybrid speciation, despite the relatively slow build-up of genetic incompatibilities between two taxa. If barrier loci are more strongly associated or recruited, it is less likely that recombination will occur and ecological barriers between taxa could be enhanced (Abbott *et al.* 2013, Bierne *et al.* 2011).

The extent to which reproductive barriers can be enhanced or broken down when taxa are still in contact remains uncertain (Abbott *et al.* 2013). But one model has been proposed to explain speciation-with-gene-flow (Feder, Egan & Nosil 2012). Within this model, a combination of divergent selection and, to a lesser extent, divergent hitchhiking (or linkage disequilibrium; when loci are more likely or less likely to occur together than what is expected through random or independent associations) separate taxa over time. This is followed by genome hitchhiking, ultimately reducing effective migration rates of sections of the genome (Feder, Egan & Nosil 2012). It is unknown the extent to which divergent and genomic hitchhiking are important for speciation-with-gene-flow. It is possible that these differences are due to measurement of these effects, a problem with the theoretical models, or general variation between speciation events (Feder, Egan & Nosil 2012). Genome hitchhiking may occur early on in speciation.

Regardless of mechanism, speciation-with-gene-flow may be evident in a number of lineages. Among certain fish taxa, the lack of multigenerational recombinants past F1 (bluegill sunfish taxa, Avise, Saunders 1984; species of trout, Leary, Allendorf & Forbes 1993) indicates pre-and post-zygotic barriers to hybridization. Similarly, rapidly-forming hybrid zones among common sculpins in the Rhine have also been instrumental in understanding local adaptation preventing admixture since, in this case, pre- and postzygotic reproductive isolation did not seem to occur (Nolte, Freyhof & Tautz 2006).

HYBRIDS MAY HAVE INCREASED OR REDUCED FITNESS RELATIVE TO PARENTS

Hybrids, or their offspring, may have increased or decreased fitness relative to the parents. In the case of increased fitness, these recombinants may out-compete the parents, or expand into a novel or intermediate habitats previously uninhabited by the parents, to which they are better suited (see Lewontin 1966). Similarly, introgression of more or fewer fit traits (alleles) to either parent population may occur (Arnold, Martin 2009, Arnold 1992). Through introgression, the selection

process has been demonstrated frequently, with combinations of genes and traits that are beneficial being selected for, and more successfully introgressed into another population than those that are harmful (Lewontin 1966).

Already within this chapter, increased hybrid fitness in extreme environments has been summarised for the diploid hybrid plant species of *Helianthus* (sunflowers), indicating positive selection for adaptive transgressive traits not found in either parent (Rieseberg *et al.* 2003). Among animals, fruit flies have been particularly informative regarding the adaptive benefits of hybrids and hybrid traits. They are relatively easy and economical to breed and control, and have wild, hybridizing taxa with which to compare them. Lewontin (1966) demonstrated the possibility of introgression between *Dacus tryoni* and *D. neohumeralis* (Queensland fruit flies), as an explanation for range expansion by *D. tryoni* southward in Australia, given that neither population extended southward before introgression. Laboratory experiments showed that hybrid samples could survive better at extreme temperatures than either parent sample, due to physiological changes brought about through an increase in genetic variation. Contrarily, experimental studies using interspecific *Drosophila* (another genus of fruit fly) hybrids showed that hybrid phenotypes are not always adaptive, and that, over multiple generations, hybrid lineages may phenotypically resemble parents when exposed to extreme environments and thereby not affect evolutionary rates (Hercus, Hoffmann 1999). Regardless, hybridization allows for a boost in the genetic variation within a population. The genetic combinations that result from hybridization may allow for extreme morphologies and phenotypes that could further lead to the exploitation of niches that are unavailable to either parent species (Mallet 2007, Abbott *et al.* 2013).

This is also demonstrated in Darwin's finches, discussed above (reduction in variation between taxa). Species of Darwin's finches (*Geospiza scadens* and *G. fortis*) on the Galápagos island of Daphne Major show high levels of hybridization, with stable hybrid zones, and where hybrids often displayed fitness levels exceeding that of parents (Grant, Grant 1992, Grant, Grant 1996, Grant, Grant 2002). These hybrids were morphologically intermediate to the parents, in both overall size and shape, increasing genetic and morphological variation relative to parent taxa (Grant, Grant 1994). Furthermore, the beak morphology (and, consequently, diet) of F1 and backcrossed hybrid finches was intermediate to the parental species, contributing to high survival rates and increased levels of fitness (Grant, Grant 1996). Further discoveries show that introgressive hybridization played a role in the adaptive radiation that characterizes Darwin's Galápagos warbler finches in the Galápagos today (Grant, Grant & Petren 2005). Similar increase in morphological variation, which may lead to adaptive radiation, was also seen in hybridized cichlid fish (Stelkens *et al.* 2009).

Among primates, some baboon hybrids appear to be relatively successful. *Papio anubis* shares hybrid zones in Ethiopia with *P. hamadryas* (Bergman, Phillips-Conroy & Jolly 2008, Bergman, Beehner 2004). These hybrid baboons exhibit mixed social organization, containing elements of mating behaviour and social structure that resemble both parent groups. Despite hybrid male behaviour being unlike that of parents, they appeared to be reproductively viable (Bergman, Phillips-Conroy & Jolly 2008). Among *P. cynocephalus* and *P. anubis* hybrids, population growth has increased, albeit with apparent asymmetry (possibly due to differences in life-history between hybrid males and one of the parents; Tung *et al.* 2008). Hybridization among primates and their hybrid zones will be more thoroughly fleshed out in the next chapter.

In the case of decreased fitness, a "hybrid sink" may attract pests and diseases, leaving the parent populations to thrive (Barton 1980). In both scenarios (increased and decreased hybrid fitness), hybridization and introgression can be selected for (or, minimally, not significantly reinforce reproductive barriers between taxa; Arnold 1992, Barton 1980). A balance between hybridization and selection may then allow for the maintenance of hybrid zones and hybridization. When a balance is not achieved, it is also possible for one or both parent species to go extinct. At a population level, hybrids typically form less than 0.1% of individuals (Mallet 2007, Mallet 2005). However, even moderately strong barriers (genetic or physical) to hybridization may allow for gene flow over hundreds of generations (Barton, Hewitt 1985).

The effects of hybridization among mice taxa are highly variable and provide some of the best studied models for hybrid zones among mammals, including the sometimes duplicitous effects of reduced hybrid fitness. Among *Mus musculus* subspecies (although it must be noted that specific, or sub-specific status is not universally acknowledged; see Carleton, Musser 2005), two hybrid zones are well known and understood. The best studied is the fairly narrow hybrid zone between *M. m. musculus* and *M. m. domesticus* across Europe (from Denmark to the Black Sea) and has been an incredibly informative model for understanding specific concepts which could arise in hybrid zones (as was briefly discussed earlier; Boursot *et al.* 1993, Shurtliff 2013). For instance, despite evidence for Haldane's Rule, reduced female fertility and selection against the introgression of sex chromosomes, gene flow still occurs across the hybrid zone (Teeter *et al.* 2010, Tucker *et al.* 1992, Teeter *et al.* 2008, Vanlerberghe *et al.* 1988, Vanlerberghe *et al.* 1986). Also adding to reduced hybrid fitness, hybrids are particularly sensitive to pests (disease), providing an interesting model for the effects of a "hybrid sink", where hybrid unfitness prevents gene flow outside of the hybrid zone (Sage *et al.* 1986, Moulia *et al.* 1993, Moulia *et al.* 1991). The similarities in width of the hybrid zone and patterns of introgression across the zone, suggests that this zone is more akin to a "tension

zone": maintained, in part, by selection (Boursot *et al.* 1993, Dod *et al.* 1993, Barton, Hewitt 1985, Dod *et al.* 2005). Introgression within this zone is also typically asymmetric (gene flow from *M. m. domesticus* into *M. m. musculus*), likely partly due to stronger conspecific preference in *M. m. musculus* females (Dod *et al.* 1993, Smadja, Ganem 2005).

Among primates, it appears as though some hybrids have reduced fitness relative to parents: from unusual, mixed appearances to uncoordinated mating systems or social structure. For instance, the hybrid zone of two macaque species (*Macaca maura* and *M. tonkeana*) is particularly narrow, with hybridization appearing to be highly affected by differences in macaque behaviour (Evans, Supriatna & Melnick 2001). Similarly, analyses of hybrid/mixed groups between *Alouata caraya* and *A. clamitans* (howler monkeys) suggest lower viability than parents and the possible effect of Haldane's rule (Aguiar, Pie & Passos 2008).

HYBRIDIZATION MAY LEAD TO A NEW HYBRID TAXON

Finally, hybridization may lead to the formation of a hybrid species, distinct from the parent taxa (Arnold 1992, Mallet 2007). Individual hybrids are often only a very small proportion of a taxon's population, and are more likely to have fitness disadvantages due to the merging of co-evolving genomes of the parent taxa. Despite this, hybrid species do occur in nature (Mallet 2007). These "hopeful monsters" (a term coined by Goldschmidt- 1940- and used by Gould- 1977, albeit not necessarily in reference to hybrids) may even accommodate theories that involve saltational (non-gradual) evolution, by acting on multiple loci and causing large-scale phenotypic changes (Mallet 2005, Dittrich-Reed, Fitzpatrick 2013). While this occurs in plants (self-fertilizing in particular) frequently through allopolyploidy, mammals are more likely to exhibit homoploid hybridization, since polyploidy speciation is incredibly rare in animals (Otto, Whitton 2000). Homoploid hybrid speciation may occur when novel combinations of alleles of the parent taxa are maintained, and reproductive barriers result between the new hybrid taxon and both parent taxa (Abbott, Albach *et al.* 2013). This is most likely if hybrids are more capable of surviving an environmental niche not available to either parent species, otherwise hybrids may not survive competition or gene flow from the parent species (Rieseberg, Archer *et al.* 1999).

The production of hybrid swarms (for instance, among subspecies of bluegill sunfish: Avise *et al.* 1984; among mules deers: Latch *et al.* 2011; or among multiple primate taxa: Arnold 1992, Arnold, Meyer 2006, Arnold 2009), which could ultimately outcompete or overwhelm parental populations, is one way in which hybrid species may be produced. Hybridizing taxa of common sculpin (fish) in

Netherlands have produced multiple lineages which may, ultimately, form hybrid taxa (Stemshorn *et al.* 2011).

Among mammals, only a few hybrid taxa have been studied or discovered, but this number is growing with greater understanding and more extensive genomic analyses (Shurtliff 2013, Yonekawa *et al.* 1988, Yonekawa *et al.* 2012, Ackermann, Bishop 2010). In the house mouse, the hybrid zone in Japan, between *M. m. musculus* and *M. m. castaneus*, is extensive and gradual, and has resulted in a new hybrid taxon, *M. m. molossinus*, one of the first, and for a long time, the only, examples of the production of a new hybrid taxon in mammals (Boursot *et al.* 1993, Yonekawa *et al.* 2012, Yonekawa *et al.* 1988, Shurtliff 2013). Secondary contact in this hybrid zone was largely anthropogenic: commensalism with sedentary humans allowed for the expansion of *M. musculus* subspecies from the Fertile Crescent, and *M. m. molossinus*, in particular, thrives in human-dominated regions.

In primates, several species are a product of hybridization: *Trachypithecus pileatus* (the capped langur), *Macaca arctoides* (stump-tailed macaque) and *Macaca munzala* (the Arunachal macaque; Ackermann, Bishop 2010, Ackermann 2010, Chakraborty *et al.* 2007, Osterholz, Walter & Roos 2008, Tosi, Morales & Melnick 2000, Zinner, Arnold & Roos 2009). Phylogenetic research on the recently discovered kipunji (*Rungwecebus kipunji*) from Tanzania indicated mtDNA that was likely inherited from yellow baboons (*Papio cynocephalus*) around 350kya, yet was morphologically similar to mangabeys (Zinner *et al.* 2009, Zinner, Arnold & Roos 2011, Burrell *et al.* 2009). Further research suggested that the kipunji itself was the result of *Papio-Lophocebus* hybridization around 0.65Ma (Burrell *et al.* 2009). However, the discovery and research of a second *Rungwecebus* population supports the genus as a sister-clade to *Papio* with localised mtDNA introgression from yellow baboons into the original population (Roberts *et al.* 2010). Similarly, morphological and genetic evidence suggests *Gorilla beringei graueri* may have arisen as a hybrid taxon through west to east gene flow (Ackermann, Bishop 2010), although other studies have failed to prove this hypothesis (Tocheri *et al.* 2016).

SUMMARY

Hybridization and, subsequently, introgression and gene flow, have profound effects regarding reproductive success and fitness among hybridizing lineages. Within this chapter, it is highlighted that genetic incongruency is observed within and among numerous taxa, influencing the evolution of

these taxa both in modern times and in the more distant past. While it is commonly understood that hybridization prevents speciation and facilitates the "merging" of divergent lineages, there are numerous examples where the results are far more complex. Hybridization is the first step towards adaptive introgression, facilitating the evolution of parent taxa. It is also possible that hybrids may thrive in intermediate environments and, themselves, form a diverging lineage.

While the evolutionary effects of hybridization have been unpacked, there are also a range of potential morphological effects, which may, in turn, affect the evolutionary trajectory of a given taxon. This will be expanded upon in the following chapter.

Some were the figures of well-known myth—gorgons, chimaeras, dragons, cyclops, and all their shuddersome congeners. Others were drawn from darker and more furtively whispered cycles of subterranean legend—black, formless Tsathoggua, many-tentacled Cthulhu, proboscidian Chaugnar Faugn, and other rumoured blasphemies from forbidden books.

H.P. Lovecraft

CHAPTER 3

HYBRID ANIMAL MORPHOLOGY

Genetic information is incredibly reliable for identifying and thoroughly examining hybridization among various taxa. Unfortunately, genetic information is not always possible to retrieve, particularly in the case of fossils or ancient populations. For this we need information on the phenotype, and especially the skeleton. Additionally, a more thorough understanding of hybrid phenotypes will help us further understand potential selective advantages or disadvantages that may result from hybridization. However, there are few studies compiling and comparing hybrid phenotypes. Fewer still look at skeletal information, which may be extrapolated onto fossil records (but see Ackermann, Brink *et al.* 2010, Ackermann, Schroeder *et al.* 2014, Ackermann, Rogers *et al.* 2006, Ackermann 2010, Ackermann, Bishop 2010, Ackermann 2009, Eichel 2014, Cheverud, Jacobs *et al.* 1993).

Hybrids do not necessarily conform, phenotypically speaking, to expectations. In many instances, hybrids are assumed to resemble a mixture between the parent populations, as was argued for

suggested hybrid hominin specimens, such as the Lagar Velho child (Duarte, Mauricio *et al.* 1999), but this is not always the case. Novel phenotypes are relatively prevalent within F1 hybrids, with some exhibiting extreme size relative to the parents, unusual colours and shapes (e.g. in the case of orchid hybrids; Rolfe, Hurst 1909), and adaptations to survive extreme environments (Dittrich-Reed, Fitzpatrick 2013). In general, hybrids exhibit a large range of phenotypic variability, with individuals either resembling one parent, a mixture of the two parents or even exhibiting extreme (transgressive) phenotypes, or range of unusual traits, unseen or rare in either of the parent populations (Ackermann, Bishop 2010, Stelkens, Seehausen 2009, Rieseberg, Archer *et al.* 1999).

THE MORPHOLOGY OF ANIMAL HYBRIDS

OBSERVED ANIMAL HYBRID MORPHOLOGIES: TRANSGRESSIVE PHENOTYPES

One notable effect of hybridization is where the hybrids display morphological traits outside of those seen in the parents, or are more likely to exhibit unusual or rare traits in the parents. Such features are considered "transgressive", and have been alluded to in the previous chapter. High frequency of unusual traits is termed "transgressive segregation" and is relatively common in hybridizing animal taxa (Stelkens, Seehausen 2009, Rieseberg, Archer *et al.* 1999). Rieseberg and colleagues (1999) reported that 78% of hybridizing animal species studied showed transgressive segregation, with 31% of the traits observed being transgressive (Rieseberg, Archer *et al.* 1999). In animals, 26% of morphological traits were transgressive within hybrids (Rieseberg, Archer *et al.* 1999), with behaviour, life-history and general physiological traits were likely to be transgressive than general anatomical traits. Furthermore, hybrids of domesticated animals (i.e. largely inbred) were more likely to exhibit transgressive traits (45% of taxa) than wild animal hybrids (24%; Rieseberg, Archer *et al.* 1999). Stelkens and Seehausen (2009) showed that out of 15 animal studies, 29% of traits were transgressive (Stelkens, Seehausen 2009), with increasing hybrid transgression with greater phylogenetic distance (Stelkens, Seehausen 2009, Stelkens, Schmid *et al.* 2009): in cichlids, this trend was particularly acute in F2s (2nd generation offspring).

How hybridization affects the skeletal and dental morphology of mammals is important for understanding ancient introgression in the past. Hybrid non-metric morphological trait variation may differ between parent and hybrid populations, with unusual and significantly high levels of supernumerary teeth (bilaterally expressed), sutural anomalies, abnormal dental crowding, and greater variation in coat/pelage colour and patterns (Ackermann, Bishop 2010, Aguiar, Pie *et al.*

2008, Freedman 1963, Ackermann, Brink *et al.* 2010, Ackermann 2010). Some of these features may preserve or fossilize after death. For instance, Goodwin (1998) compared bilateral supernumerary distal upper molars in extant and fossil ground squirrel colonies (*Spermophilus richardsonii* and *S. elegans*). In extant samples, specimens exhibiting the trait were significantly more likely to come from areas with known hybrids. Such a trait, observed in Pleistocene ground squirrel specimens, adds support to the assertion that ancient hybridization occurred among ground squirrels in the Pleistocene. Hybrids between the blue and black wildebeest show a number of morphological anomalies, including a unilateral rotated premolar, unusual premolar roots, and unusual sutural anomalies (particularly additional sutures in the premaxillary region) which were not observed in non-hybridized samples (Brink 2005, Ackermann, Brink *et al.* 2010). Furthermore, intermediate horn morphology and substantial cranial variation can also be used to detect these hybrids. Intermediate and unusual dental traits, combined with large cranial size, have also been put forward as evidence that an anomalous cetacean skull is that of a narwhal-beluga hybrid (Heide-Jørgensen, Reeves 1993). Further examples of skeletal morphological traits present in a handful of primate hybrid studies, will be discussed later in this chapter.

Observed animal hybrid Morphologies: Variation and Asymmetry

Extensive research has been conducted on hybridization in aquatic animals, supporting a hypothesis of large variability in hybrid morphologies among different groups. Transgressive features (outside of parental variation) and novel phenotypes occur in cichlid fish hybrids, with genetic distance between taxa explaining between 52% of transgressive (in this instance, body shape) frequency in F1 hybrids, and 78% in F2 hybrids (Stelkens, Seehausen 2009, Stelkens, Schmid *et al.* 2009). This supports the idea that hybridization facilitates the rapid production of phenotypic variation. In a group of North American minnows (genus *Gila*), hybridization and introgression throughout their evolutionary history has led to great morphological diversity (Dowling, DeMarais 1993). Valentin and colleagues (2002) showed that a parental body shape occurred in hybrids of sympatric species of redfish, although they were more variable. Hybridization in African cichlids is direct evidence for the role of hybridization in creating and maintaining morphological variation that may be adaptive, where hybrids (particularly of second generation) show increased diversity and relaxed biological restraints on covariation (Selz, Lucek *et al.* 2014, Stelkens, Schmid *et al.* 2009). Among individuals of a hybrid population of treefrogs, fluctuating asymmetry was comparable among all but one cranial measurement (which was, in fact, lower in F1 hybrids) between parental and various hybrid individuals (Lamb, Avise 1986).

Mice are excellent models for mammalian hybridization effects, as are used within this thesis. Researchers have looked at wild-type house mouse hybrids in order to understand the effects of hybridization on mouse morphology in both laboratory controlled crosses and across hybrid zones (with a focus mainly on the intra-specific mouse hybrid zone in Europe). Laboratory-controlled crosses between *M. m. musculus* and *M. m. domesticus* indicate that hybrids have heterotic mandibular size (more significant in F1s) and increased variance (particularly in F2 recombinants) compared with parent strains, but a generally intermediate mandibular shape (Renaud, Alibert *et al.* 2009, Renaud, Alibert *et al.* 2012). In studies looking at modularity of the mandible, the effects of heterosis were variable: some modules were additive (intermediate between parents), some parental (dominant), and some transgressive, which may ultimately be interpreted as a novel phenotype (Renaud, Alibert *et al.* 2012). Alibert and colleagues (1997) discovered these mouse hybrids (both F1 and F2 and backcrossed recombinants) were larger than expected in tooth size (i.e. larger than mid-parental value). Studies looking at wild mice across a Danish hybrid zone noted that skull and mandibular shape change was transitional and correlated with allozymic introgression and percentage of mixed genotypes across the zone (Auffray, Alibert *et al.* 1996, Pallares Amaya 2015). Similarly, cranial qualitative data showed a steep gradient of intermediate features across the Balkans hybrid zone although a small proportion of introgressed features were detected 100km from the hybrid zone (Macholán, Kryštufek *et al.* 2003).

Fluctuating asymmetry (FA) has also been studied in mice, and the lack of it has often been interpreted to represent developmental stability and an increase in fitness (Alibert, Fel-Clair *et al.* 1997). A decrease in mandibular shape and tooth size FA in F1 hybrid mandibles, and F1 and recombinants (backcrosses and F2s) of laboratory-controlled *M. m. musculus* and *M. m. domesticus* was detected (Renaud, Alibert *et al.* 2009, Alibert, Fel-Clair *et al.* 1997). Similarly, a decrease in FA of molar width and length towards the centre of the *M. m. musculus/M. m. domesticus* hybrid zone in Denmark, albeit not as low as detected in laboratory strains, supports these trends in natural populations as well (Alibert, Renaud *et al.* 1994, Alibert, Fel-Clair *et al.* 1997). This apparent hybrid vigour is in contrast to research that has shown dysgenesis in these hybrids in terms of reproductive fitness and parasite load (Vanlerberghe, Boursot *et al.* 1988, Tucker, Sage *et al.* 1992, Vanlerberghe, Dod *et al.* 1986, Dod, Jermiin *et al.* 1993, Moulia, Aussel *et al.* 1991). It is worth noting that FA is positively correlated with inbreeding, and negatively correlated with allozyme/allelic heterozygosity in both laboratory and wild populations (Alibert, Fel-Clair *et al.* 1997, Leamy 1992, Leamy 1984).

The link between greater developmental stability (brought about by genomic coadaptation) and heterozygosity has been made by multiple authors, with particularly supportive examples from intra-

populational studies of inbreeding depression in *Drosophila* (Biémont 1983), the rainbow trout (Leary, Allendorf *et al.* 1983) and in mice (Alibert, Fel-Clair *et al.* 1997). There are also large discrepancies in the patterns of increased variance, lower FA and higher developmental stability, which have been demonstrated in hybrid mice dentition and mandibles (Renaud, Alibert *et al.* 2009, Alibert, Fel-Clair *et al.* 1997, Alibert, Renaud *et al.* 1994), but not in other animals, such as *Drosophila* (Alibert, Auffray 2003, Rego, Matos *et al.* 2006). These results also contradict other research on the dorsal skull of hybrids of laboratory mice, which shows only shape but no size FA (Debat, Alibert *et al.* 2000), and no apparent FA of the ventral skull, or mandibular size towards the centre of the hybrid zone in Central Europe (Mikula, Macholán 2008, Mikula, Auffray *et al.* 2010). Contrarily, FA in mandibular shape did decrease significantly towards the centre of the hybrid zone in Central Europe, highlighting that decrease in developmental stability, as measured by FA, may be due to overdominance (heterozygote advantage; Mikula, Aufray *et al.* 2010).

Genetic/Developmental Underpinnings for transgression

Novel or transgressive traits are often the result of a new combination of alleles and that are more or less fit. These are subject to selection processes, with some adaptive potential (Abbott, Albach *et al.* 2013, Stelkens, Seehausen 2009). Transgressive hybrids with higher fitness levels than the parent taxa in novel or intermediate environments may then diverge from the parent taxa (Dittrich-Reed, Fitzpatrick 2013). As indicated in chapter 2, transgressive hybrids have, therefore, been referred to as “hopeful monsters”, reviving the theory of evolutionary novelty on which natural selection may act (Dittrich-Reed, Fitzpatrick 2013, Mallet 2007, Goldschmidt 1940). This also offers a potential scenario under which hybrid speciation may occur.

A general rule was that the greater the genetic (and, presumably, temporal) divergence between two animal taxa, the more likely transgressive phenotypes are to arise (Abbott, Albach *et al.* 2013, Stelkens, Seehausen 2009, Rieseberg, Archer *et al.* 1999). However, Stelkens and colleagues (2009) found this correlation between transgressive frequency and genetic distance to be more linear in F2 recombinants than F1s. Rieseberg and colleagues (1999) identified that wild animal intraspecific hybrids (79%) were more likely to exhibit these novel traits, than interspecific hybrids (25%). Similarities between parent species may therefore be more important at determining transgression in hybrids (Rieseberg, Archer *et al.* 1999, Stelkens, Seehausen 2009). It is worth noting that in this study, only 26% of morphological traits studied were transgressive in animal hybrids (many were behavioural or life-history traits).

The novel phenotypic traits exhibited by many hybrids have important genetic underlying mechanisms. In the case of extreme phenotypes, additive alleles in the two parent taxa may lie in different loci along the genome, but could generate extreme hybrids if combined appropriately (Abbott, Albach *et al.* 2013, Stelkens, Seehausen 2009). These quantitative trait loci (QTL), fixed in either parent population, recombine in the hybrids to form a complementary gene action, generating extreme phenotypes (Rieseberg, Raymond *et al.* 2003, Stelkens, Seehausen 2009). These complementing alleles may then be responsible for transgressive segregation seen in some hybrids (Stelkens, Seehausen 2009). In plants, complementary gene action has been shown to be the primary cause for transgression; although overdominance (alleles at a particular locus creating more extreme values in heterozygotes than homozygotes) and epistatis (interaction of genes from separate loci) may also be relevant causes (Stelkens, Seehausen 2009, Rieseberg, Archer *et al.* 1999). With regards to complementary gene action, species which are more phenotypically similar to each other are more likely to produce transgressive hybrid traits due to stabilizing selection on different alleles at certain QTLs (Stelkens, Seehausen 2009). Complementary gene action may then not occur when parent species are too dissimilar.

Other explanations for transgression in populations with hybrids include an elevated mutation rate in hybrids compared with parent species. Variability in chromosome number between parent species could also contribute to this where relevant (Rieseberg, Archer *et al.* 1999). This, however, is much less likely due to the rarity of chromosome number variation between hybridizing populations, the highly heritable nature of transgressive phenotypes and the reproducibility of transgressive traits (Lewontin 1966). Epistasis and complementary gene action are therefore the most likely genetic causes of novel phenotypes in animals (Stelkens, Seehausen 2009, Rieseberg, Archer *et al.* 1999). Since complementary gene action requires the fixing of genetic differences between populations, the high proportion of hybrids between inbred (and/or domestic) populations exhibiting transgressive phenotypes are unsurprising (Rieseberg, Archer *et al.* 1999). Hybrids between these inbred populations are also possibly developmentally less stable, considering the opportunity for rare recessive alleles to be revealed is likely to be greater following a founding effect (Ackermann 2007).

These explanations may be used to explain hybrid transgression which remains fixed, or is selected for, in a population. Under the Bateson Model, which allows for transgressive phenotypes (Dittrich-Reed, Fitzpatrick 2013), different genetic mutations occur in two diverging populations. However, due to stabilizing evolution, these may still result in similar phenotypes in these populations. When hybridization occurs, these mutations combine, possibly expressing a new phenotype. Even in the

light of reduced hybrid fitness or increased sterility caused by the combination of other genes, recombination could still allow for the selection of transgressive phenotypes in multigenerational recombinants or backcrossed individuals. Thus transgressive phenotypes resulting from hybridization may even be selected for over time.

Hybrid phenotypes may also arise from genome restructuring, different levels of hormone release and the timing of gene expression through divergence of regulatory genes (Landry, Hartl *et al.* 2007), duplication and deletion of genes (Nei, Nozawa 2011) and even epigenetic effects (Michalak 2009). Supernumerary dentition (as seen in baboons and ground squirrel hybrids) could be indicative of a breakdown in coordination during development due to the mixing of separately evolved genomes (Ackermann 2007). The forced or inhibited activation of key genes may stimulate hyperactivity progenitor cells, causing tooth row elongation or the splitting of the tooth germ (Ackermann, Rogers *et al.* 2006, Wang, Fan 2011, Goodwin 1998, Ackermann 2007). Such epigenetic effects better explain traits which are more common in hybrids of numerous different taxa, since it does not rely on the same genetic mutations to occur and recombine to produce similar traits across.

PRIMATE HYBRID MORPHOLOGIES

An understanding of hybridization in primates is important for better understanding hybridization among hominins in the past (Jolly 2001, Ackermann, Schroeder *et al.* 2014, Ackermann, Rogers *et al.* 2006, Ackermann 2010, Ackermann, Bishop 2010). In part this is due to them being closely related to us, and in part due to many of these species occupying similar geographic niches which these hominins may have occupied. For instance, baboons make particularly useful analogues for gene flow in Pleistocene hominin evolution, being more diverse than extant humans or apes and having potentially similar population structures as past hominins (Jolly 2001, Ackermann, Rogers *et al.* 2006). Baboons readily hybridize amongst each other in contact regions, despite some lineages having recent common ancestry up to 1.8 Ma. This is comparable to the time of the emergence of the genus *Homo*, implying that such a divergence time alone is not an effective barrier against hybridization of members within our genus (Holliday et al. 2014).

External morphologies

While evidence for reticulation is abundant among primate species, documentation of primate hybrid morphology is limited. The vast majority of research in primate hybrid zones looks at the external phenotype (which is not easily extrapolated onto the skeleton) or measurements such as weight and overall body size. However, it is important to evaluate these traits, the potential connections which they may have with the skeletal morphology, and the ways in which they influence hybrid success and showcase hybrid zones among primate taxa today. External morphological diversity is often the first line of evidence for hybridization among primate taxa. Here, we will explore the variety of primate hybrids while also looking at the morphological indicators of hybridization among taxa.

Before summarising the literature, it is worth pointing out that the taxonomic understanding of primates (particularly lemurs) has changed significantly within the past two decades. In order to make referring to the literature easier, the names used within the texts are those used in the literature cited, and changes will be briefly referred to or mentioned in parentheses.

Malagasy lemurs exhibit high levels of species richness despite living in a comparatively small geographic space (Arnold, Meyer 2006, Wyner, Johnson *et al.* 2002). Considering hybridization has been shown to play a role in rapid diversification in numerous taxa, it is hypothesized that hybridization may have contributed to the diversity and radiation of Malagasy lemurs (Pastorini, Zaramody *et al.* 2009). Indeed, hybridization among lemurs is common. Mixed pelage patterns or intermediate external morphologies have long been used to identify hybrids. Researchers first observed a lemur exhibiting intermediate or mixed pelage colouration between wild mongoose (*E. mongoz*) and brown (*E. fulvus*) lemurs, indicating lemur interspecific hybridization (Curtis, Zaramody 1998, Wyner, Johnson *et al.* 2002). Pelage colouration in these hybrids was complex, with F1 and backcrossed hybrids exhibiting intermediate pelage colouration, and F2s and further recombinants more closely resembling parents (Pastorini, Zaramody *et al.* 2009). This has since been supported by genetic studies which have shown that bidirectional introgression between the two species does occur, and, furthermore, that male F1 hybrids could be fertile (Pastorini, Zaramody *et al.* 2009, Zaramody, Pastorini 2001).

Delmore and colleagues (2013) investigated a stable, extensive interspecific hybrid zone between *Eulemur rufifrons* (red-fronted) and *E. cinereiceps* (grey-headed lemur; Delmore, Brenneman *et al.* 2013, Delmore, Louis *et al.* 2011). Here, hybrids exhibit intermediate or mosaic-parental pelage patterns along the hybrid zone (which were successful at identifying hybrids with varying levels of introgression, corresponding with genetic markers). Male hybrids also had longer tails than either

parent, a transgressive train possibly resulting from differences in sexual selection between parents (Delmore, Louis *et al.* 2011). Among mongoose lemurs, the black lemur (*E. mongoz macaco)* and the Sclater's lemur *(E. m. flavifrons)*, also hybridize, indicated by intermediate external phenotypes (Meyers, Ratsirarson 1989, Rabarivola, Meyers *et al.* 1991). An extensive interspecific hybrid zone also occurs between black-and-white (*Varencia variegate*) and red (*V. rubra*) ruffed lemurs (Vasey, Tattersall 2002). Furthermore, intraspecific hybrid zones also occur between a number of brown lemur subspecies: grey-headed brown (*E. cinereiceps*, previously white-collared: *E. fulvis albocollaris*) and red-fronted lemurs (*E. f. rufus*; Wyner, Johnson *et al.* 2002); and between *E. f. fulvus* and *E.f. rufus* (Lehman, Wright 2000). It is worth noting, however, that *E. fulvus* "subspecies" are since argued to be separate species (Mittermeier et al. 2010).

Hybridization has also been observed among numerous New World Monkey taxa. For example, discordance between phylogenetic data, morphology (pelage patterns in particular) and geographic patterning in spider monkeys indicate varying rates of introgression for different parts of the genome. This "semi-permeable boundary" is possibly due to varying rates of selection and drift (Key 1968, Collins, Dubach 2001, Collins, Dubach 2000, Morales-Jimenez, Disotell *et al.* 2015). While incomplete lineage sorting may explain this discordance, it is likely that, among such closely related taxa, differential levels of introgression occurred (Arnold, Meyer 2006). Furthermore, in captivity, a viable male F1 hybrid resulted from the pairing of a red and black spider monkey (*Ateles geoffroyi panamensis* and *A. fusciceps robustus*), with intermediate pelage colouration (Rossan, Baerg 1977). Similar such hybrids between the two species have been identified in the wild (Rossan, Baerg 1977).

A similar discordance amongst phylogenies based on nuclear and mitochondrial genomes in howler monkeys supports a scenario of ancient gene flow between species and among subspecies (Cortés-Ortiz, Bermingham *et al.* 2003, Arnold, Meyer 2006, Gregorin 2006). Furthermore, contemporary hybrids between howler monkey species do occur. Mixed troops comprised of *Alouatta palliata* and *A. pigra* hybrids have been argued to represent a secondary contact zone. However, these hybrids appear to exhibit male infertility (Cortés-Ortiz, Duda *et al.* 2007). These taxa, which diverged approximately 3 Ma, produce hybrids which differ in size from parental groups, with male hybrids especially being large (transgressive or heterotic) relative to both parental taxa (Cortés-Ortiz, Bermingham *et al.* 2003, Cortés-Ortiz, Duda *et al.* 2007, Kelaita, Cortés-Ortiz 2009). The male infertility may explain the rarity of F2 hybrids. Other multigenerational recombinants closely resemble the parents, and are thus "invisible" without genomic evidence, despite evidence for backcrossing and mixed groups in the wild (Kelaita, Cortés-Ortiz 2009). Similar evidence for sympatry and mosaic pelage patterning in potential hybrid individuals supports the scenario of

hybridization between *Alouatta caraya* and *A. clamitans* in Brazil (Aguiar, Mellek *et al.* 2007, Aguiar, Pie *et al.* 2008), and it is also likely hybridization occurred with lower viability, following Haldane's Law. In this scenario, hybridization is associated with a high level of colour configurations and pelage patterning (Aguiar, Pie *et al.* 2008).

Also in Brazil, in an ancient natural hybrid zone between *Callithrix penicillata* and C*. jacchus* (black-tufted and common marmosets), more genetic diversity was found than in an anthropogenic hybrid zone. Furthermore, the formation of hybrid swarms occurs in the more contemporary hybrid zone (Malukiewicz, Boere *et al.* 2014). *C. penicillata* and *C. geoffroyi*, other Brazilian marmoset taxa, also hybridize, with recombinants exhibiting intermediate to parental facial pelage colouring (with *C. penicillata* being particularly dominant) and various morphometric traits (such as tail length, body mass, and cranial measurements) being intermediate, parental or even transgressive (Fuzessy, de Oliveira Silva *et al.* 2014). Notably, the hybrids were not significantly smaller than the smallest parent for any of the twelve morphometric traits. Furthermore, these traits appeared to be uncoupled, supporting the idea that hybrid transgressive phenotypes, or novelty, may itself be the result of fewer constraints on the integration of hybrid morphologies, as was discussed in relation to mice and cichlids earlier in this chapter (Fuzessy, de Oliveira Silva *et al.* 2014, Renaud, Alibert *et al.* 2012, Parsons, Son *et al.* 2011). Furthermore, genetic incongruence of some marmoset and tamarin taxa complicates the reconstruction of some phylogenetic relationships. This may be explained by past introgression (Arnold, Meyer 2006, Tagliaro, Schneider *et al.* 1997, Peres, Patton *et al.* 1996, Cropp, Larson *et al.* 1999, Matauschek, Roos *et al.* 2011). Analyses of cranial and skeletal differences between hybrid and parent tamarins will be discussed below.

In Old World monkeys, baboons, macaques, and guenons provide the clearest and certainly best-studied evidence of hybrid morphologies. Baboons are a complex phylogenetic group, with incongruent phylo-geographic patterning that hints at ancient hybridization, possibly accelerated by expanding and shrinking habitats during Pleistocene glacial cycling (Zinner, Arnold *et al.* 2011, Zinner, Groeneveld *et al.* 2009, Wildman, Bergman *et al.* 2004). Hybrid zones are well known between baboon species (Tung, Charpentier *et al.* 2008, Bergman, Beehner 2004, Alberts, Altmann 2001), and are relatively well-studied, with much attention placed on the morphological variation of populations in these zones. Examinations of phenotypic traits in baboon hybrids, including coat (and muzzle) colour, hair length, body shape (including aspects of size), head shape, tail length and bend, have revealed clinal variation exists between the parental taxa in both the Awash baboon hybrid region (*P. anubis* x *P. hamadryas*) and baboon hybrids in Amboseli, Kenya (*P. anubis* x *P. cynocephalus*; Alberts, Altmann 2001). Although the Awash hybrid males tend to display a more

bimodal distribution of traits, with a leaning towards one phenotype or the other (Phillips-Conroy, Jolly 1986, Phillips-Conroy, Jolly *et al.* 1991), the Amboseli hybrids displayed a continuous range of morphological variation ranging from Anubis to yellow (*cynocephalus*; Alberts and Altmann, 2001).

Among macaques, hybrid zones occur between *M. mulatta* and *M. fascicularis* (Fooden 1964), *M. tokeana* and *M. hecki* (Bynum, Bynum *et al.* 1997, Bynum 2002), and *M. maurus* and *M. tonkeana* (Schillaci, Froehlich *et al.* 2005). What is more, hybrid origins of *M. arctoides*, between *M. fascicularis* and *M. thibetana* (Tosi, Morales *et al.* 2000), and *M. munzala*, between *M. assamensis* and *M. thibetana* (Chakraborty, Ramakrishnan *et al.* 2007), have been suggested. Similar ambiguity in hybrid traits can be seen among different macaques. Hybrids between Sulawesi macaque species (*M. tonkeana* and *M. hecki*, and *M. maurus* and *M. tonkeana*) have been shown to differ morphologically from their parental taxa in terms of pelage, body size and shape, and growth allometry (Fooden 1964, Bynum, Bynum *et al.* 1997, Bynum 2002, Schillaci, Froehlich *et al.* 2005, Hamada, Urasopon *et al.* 2006). There was not a great deal of size heterosis in these hybrids (Schillaci, Froehlich *et al.* 2005). In north-eastern Thailand, Rhesus macaques (*M. mulatta*) and long-tailed macaques (*M. fascicularis*) are sympatric and also show evidence for hybridization. However, they differ from the Sulawesi macaque hybrids in more closely resembling parents: comparisons of body size/proportions in both wild and captive hybrid specimens indicate that females have small body size and relatively long tail length resembling more closely long-tailed macaques (Hamada, Urasopon *et al.* 2006, Fooden 1964). Additionally, observations of a hybrid zone between guenon species in Gombe (*Cercopithecus ascanius* and *C. mitis*) have demonstrated a range of pelage colour and pattern intermediate between the parental species in a hybrid zone (Detwiler *et al.*, 2005).

Intergeneric hybridization has also been observed among Old World Monkeys, both in captivity and in the wild (Dunbar, Dunbar 1974, Jolly, Woolley-Barker *et al.* 1997, Markarjan, Isakov *et al.* 1974, Moore, Janish *et al.* 1999). Rheboons, the hybrid between *Macaca* and *Papio* genera, obtained from the Sukhumi Monkey colony, exhibited many parental features, resembling baboons in eye colour and body build, and macaques in cranio-facial form and hair colouring (Markarjan, Isakov *et al.* 1974). Similarly, *Papio* x *Theropithecus* hybrids also exhibit suites of parental traits in form, body build and colouring. Intermediate traits were also observed, such as with the sexual skin type and ischial callosities. Similarly, a new, transgressive feature was also noted: lack of hair around the nipples of the chest. While the rheboons are non-fertile and survival is rare (Moore, Janish *et al.* 1999), *Papio* x *Theropithecus* hybrids retain fertility (Markarjan, Isakov *et al.* 1974). In the wild, three potential intergeneric hybrids have been reported between *Theropithecus gelada* and *Papio anubis* in sympatric areas in Ethiopia, more closely resembling *Theropithecus* in most of the phenotype

(similar callosities and bare skin), but exhibit *Papio*-like short, coarse, blue-grey hair and yellow tail (Dunbar, Dunbar 1974). In a more detailed examination of five *Theropithecus gelada* and *Papio hamadryas* F1 and backcrossed hybrids in Ethiopia (Jolly, Woolley-Barker *et al.* 1997), most traits examined showed an intermediate (nasal profile, mane, facial pigmentation and dental characteristics) or parental (many external sexual characteristics) phenotype, with heterotic/ transgressive (large) body size. Taken together, this evidence indicates complex patterns of morphological expression in the external phenotype of primate hybrids.

Hybridization has been identified as occurring among lesser apes (Myers, Shafer 1979, Montagu 1950, Brockelman, Srikosamatara 1984, Marshall, Sugardjito 1986). Even intergeneric crosses have been reported between a male gibbon (*Hylobates moloch*) and a female siamang (*Symphalangus syndactylus*): a Siabon, despite differing chromosomal numbers (Myers, Shafer 1979). Furthermore, there is support for ancient hybridization between *Hylobates albibarbis* and other lesser apes (Mootnick *et al.* 2010, Monda *et al.* 2007). More astonishingly, a hybrid between *Hylobates lar* (chromosome number=22) and *Nomascus leucogenys* (a sister genus to *Hylobates* and *Symphalangus*; n = 26) was described: a Larcon (Hirai et al. 2007). While both groups are referred to as gibbons (*Hylobates*), molecular studies reveal far greater mitochondrial distances than between gibbons and siamangs. Pelage characteristics of the Larcon are mixed: some (white ring around the face, and white hands and feet) diagnostic of *Hylobates lar*, some (bushy white whiskers and black crest) like males in the *Nomascus* species, and some (colouring on the back and abdomen) that appear intermediate (Hirai et al. 2007).

Comparatively little is known about hybridization among ape taxa (particularly great apes). This is likely due to few Great Ape taxa currently occurring in overlapping geographic areas. However, morphological and genetic evidence may support a scenario of gene flow in the past amongst various taxa. For instance, past introgression has been suggested for gorillas, with greater gene flow from western to eastern gorillas (Ackermann, Bishop 2010; but see Tocheri, Dommain *et al.* 2016). Thus, *Gorilla beringei graueri* is a potential taxon originating from ancient hybridization events. Similarly, despite exhibiting allopatric divergence from as far back as 1.6 Ma, researchers have detected asymmetric introgression between Bornean and Sumatran orangutans, possibly facilitated by human transfer (Kanthaswamy, Smith 2002), or sex biased dispersals due to volcanic activity (Nater *et al.* 2011). High-coverage genome analyses of *Pan* species (which diverged between 1.6-2.1 Ma) indicate that ancient gene flow from bonobos (*Pan paniscus*) into central and eastern chimpanzees (*P. troglodytes*) occurred both between 550-200 Ka, and after 200 Ka, contributing to less than 1% of the genome of some chimpanzee populations (de Manuel *et al.* 2016, Hoelzel 2016).

SKELETAL MORPHOLOGIES

Although hybrids are shown to have a suite of transgressive traits, it is important to note that many of these traits (e.g. pelage colour or texture) cannot be directly observed within the fossil record. However, differing patterns of skeletal traits have also been observed in primate hybrids relative to parents.

Cranial analyses of parents and F1 hybrids of tamarin subspecies (*Sanguinus fuscicollis illigeri* x *S. f. lagonotus* and *S. f. illigeri* x *S. f. leucogenys*) show differential results (Cheverud, Jacobs *et al.* 1993). (It is worth noting that small tamarins, such as *S. fuscicollis*, has since been placed in a new genus, Leontocebus, with the original subspecies now regarded as separate species; see Rylands et al. 2016). *S. f. illigeri* x *S. f. lagonotus* (now *S. illigeri* x *S. lagonotus*) hybrids were typically larger in cranial size than both parents or were comparable to the larger parent, displaying greater-than-midpoint values in cranial measurements. *S. f. illigeri* x *S. f. leucogenys* (now *S. illigeri* x *S. leucogenys*) hybrids also have measurements which are greater than parental midpoint, but this is not consistent (and fewer still were significant) in all cranial measurements. Similarly, greater-than-midpoint values occurred in these same subspecies crosses for 30% of examined postcranial variables, six of which were significant (Kohn, Langton *et al.* 2001). Morphological analyses have also been conducted on *Callithrix kuhlii*, a marmoset taxon argued to have originated as a hybrid between *C. geoffroyi* and *C. penicillata*. However, cranial analyses show this species is distinctive from its supposed parents, and morphologically does not resemble *C. penicillata* (Marroig, Cropp *et al.* 2004).

In contrast, larger-than-midpoint measures were not widespread among olive-yellow (*P. Anubis* x *P. cynocephalus*) baboon hybrids (Ackermann, Rogers *et al.* 2006). This is possibly due to these taxa having diverged relatively recently (350Ka; Zinner, Wertheimer *et al.* 2013), without significant cranial differentiation throughout their evolution. Yet studies of these hybrids, derived from the Southwest National Primate Research Center (SNPRC; changed from Southwest Foundation for Biomedical Research), have shown larger proportions of bilateral unusual or rare non-metric dental and cranial traits compared with their parents (Ackermann, Rogers *et al.* 2006, Ackermann, Schroeder *et al.* 2014). These include high levels of supernumerary dentition (between 31% and 50% of F1 hybrids), something also observed in ground squirrel hybrids (Ackermann, Rogers *et al.* 2006, Ackermann, Schroeder *et al.* 2014, Ackermann 2009, Goodwin 1998). These supernumerary teeth were largely mandibular, bilaterally expressed and full-sized, an unusual expression of non-metric traits in primates (Hallgrímsson, Donnabhain *et al.* 2005, Lavelle, Moore 1973, Rajab, Hamdan 2002). One known individual expressed bilateral canine duplication. Such a trait was observed in

the literature only once prior: an individual from southern Malawi (a known hybrid zone between chacma and olive baboons) expressed bilateral canine duplication and described as a hybrid (Ackermann, Rogers *et al.* 2006, Ackermann, Schroeder *et al.* 2014). Similarly, high proportions of zygomaxillary sutures, relative to parents, were also observed (Ackermann, Rogers *et al.* 2006).

This further indicates that heterosis (larger-than-midpoint measures) alone may not be a good cranial indicator of hybridization among closely related taxa, and that an understanding of phylogenetic divergence and the interplay between non-metric traits and size is necessary. In a further study, hybrid males were shown to have high levels of supernumerary teeth (mostly distomolars, and a few supernumerary premolars), females have high levels of dental crowding, and both sexes exhibit high levels of unusual sutures (additional zygomatic sutures and unusual spicules or ossicles) and remnant metopic sutures (Ackermann, Schroeder *et al.* 2014). Although supernumerary teeth were observed in F2, B1 and B3 generations of baboon hybrids (especially in males), the majority of individuals exhibiting this unusual trait were F1 hybrids (Ackermann, Schroeder *et al.* 2014); this may be affected by sample size.

Eichel (2014) continued this work on baboon hybrids by examining the nasal cavity of olive and yellow baboons, and their F1 hybrids (Eichel 2014, Eichel, Ackermann 2016). This was an important study for exploring potential human-Neanderthal hybrids in the fossil record, where both species show marked differences in the nasal region of the cranium. While non-metric traits in the nasal cavity were shown to not be significantly different between parents and hybrids, size (heterosis more specifically) and shape differences in the rhinion, choana and mid-naso-pharynx were identified.

High occurrence of rare non-metric traits in primate hybrids may not be limited to the first few generations of baboon hybrids. High frequency of similar non-metric traits (supernumerary teeth-distomolars, and more frequently in males—, rotated premolars and unusual sutural variants—*os parietale divisum*) were observed in eastern lowland gorillas- particularly *G. b. graueri* (Ackermann, Bishop 2010). A scenario of hybrid origins was therefore supported by both mitochondrial genetic evidence and morphological signatures (but see Tocheri, Dommain *et al.* 2016). This implies that these traits may continue at higher-than-expected levels long after initial hybridization took place (in this case, 80 Ka).

Although research on primates such as baboons has greatly enriched our understanding of hybridization in human evolution, there are limitations for relying on them solely. For instance, building a thorough animal model using primates, with full control over parental ancestry and breeding, is largely expensive and would take too long to achieve, with long breeding and maturing times. Where greater control over samples has been implemented (e.g. Ackermann, Rogers *et al.* 2006, Ackermann, Schroeder *et al.* 2014), the depth of research possible (for instance, the use of further recombinants, post-F1) is shallow or the sample sizes limited.

Mice make excellent model organisms, and are used extensively in medical and scientific research, the bulk of which is extrapolated or used to study human health and physiology. Some studies have also used mice to better understand the effects of another evolutionary force: selection. As discussed in this chapter and in Chapter 2, mice have also been used extensively to understand hybridization and hybrid zones, although no study currently looks at the morphological commonalities and differences among different hybrid groups. Since mice are also from a rapidly-diversifying family, with large geographic spread, and numerous species and subspecies, they are appropriate model organisms for understanding numerous scenarios for potential gene flow in human evolution.

SUMMARY

This chapter focussed on the morphological effects of hybridization in animals, focussing on primates. Transgressive and unusual features are present both in the external and skeletal morphologies of mammalian (including primate) hybrids. While genetic explanations such as complementary gene action and epistasis have been demonstrated in some hybrids, certain patterns of features common in hybrids possibly result from factors which affect the timing and release of hormones and subsequent effects development. Additionally, this may be influenced by the phylogenetic distance of the parent taxa.

Intermediate and mosaic external morphologies (pelage, size, etc.) have proven useful in detecting hybridization among numerous animal, including primate, taxa. Parental phenotypes, which may obscure hybridization detection by researchers, are present in many hybrids, particularly in multigenerational crosses. Larger-than-midpoint measurements or values appear to be fairly

common among animal hybrids, and, importantly, have been detected in the cranium of baboon and tamarin hybrids.

A better analogy for hominin macroevolution is that of a braided stream with several large, winding, channels. Some large channels are connected to each other by smaller channels. The large channels represent lineages; the smaller channels represent gene flow between them. As with almost all species that have ever existed, most of the large channels peter out, and in the end only one large channel remains—that representing our own species, Homo sapiens*.*

Holliday 2003

CHAPTER 4

HOMININ HYBRIDIZATION

Since the sequencing of the Neanderthal genome in 2010 (Green, Krause *et al.* 2010), our understanding of hybridization and resulting gene flow in the hominin fossil record has accelerated tremendously. Prior to 2010 the concern was focussed primarily on whether hybridization among late Pleistocene hominin taxa could occur at all, reflected in the dichotomous Recent African Origins (RAO) versus Multiregional Origins debate. Since 2010, research has shifted away from whether hybridization among late Pleistocene hominins occurred, to the extent and details of these hybridization events. New research has pinpointed recent multigenerational recombinants in the fossil record (Fu, Hajdinjak *et al.* 2015, Fu, Li *et al.* 2014), has identified adaptive (and maladaptive) introgressed genes from Neanderthals and other hominins into modern humans and vice versa (Abi-Rached, Jobin *et al.* 2011, Racimo, Sankararaman *et al.* 2015, Huerta-Sánchez, Jin *et al.* 2014, Racimo, Marnetto *et al.* 2016, Gittelman, Schraiber *et al.* 2016, Mendez, Watkins *et al.* 2012b, Mendez, Watkins *et al.* 2012a), and has even shown introgressed remnants in the genomes of living human populations of previously unknown (or unknowable) hominin taxa (Hammer, Woerner *et al.*

2011, Meyer, Kircher *et al.* 2012, Lachance, Vernot *et al.* 2012, Vernot, Akey 2015, Vernot, Akey 2014).

Much of this research is based on the increasingly-sophisticated field of ancient genetics. What is often missing from this research, however, is the connection to the morphology. This is important because, while the genetics has propelled our understanding of past hominin interactions, it is limited in its reach. Most of the hominin biological record is fossilized, and answering questions deeper in time, or in areas where ancient DNA is not preserved, is impossible with genomics alone. Furthermore, because our understanding of links between the human phenotype and genotype are still quite limited, the genetic evidence, for the most part, is unable to inform us about the broader effects of hybridization on hominin morphology, and how this may have affected the phenotypic evolution of our lineage. It is therefore important for us to build models for understanding the morphology of known animal hybrids. These models can be quite powerful when combined with known hominin reticulation events. This chapter will primarily focus on the evidence for hybridization among Middle to Late Pleistocene hominins, a period of intense hybridization that is becoming increasingly well-characterised. This chapter will also explore how reticulation in the more ancient fossil record or around our divergence from chimpanzees is currently understood.

UNDERSTANDING/DEBATING HYBRIDIZATION BEFORE 2010

Scientists have tried to understand the origins of human modernity since before Darwin. However, gradually increasing samples of past hominins, adequate dating techniques and advances in human genetics meant that our understanding of modern human origins – and subsequent debates – only really matured between the 1980s through to the early 2000s. Much of the discussion hinged on the polarised discourse between proponents of Recent African Origins (RAO) and the Multiregional Origins Model of modern humans (summarised in Stringer 2002, Stringer, Humphrey *et al.* 1997, Klein 1995, Klein 2008, Hawks, Cochran 2006, Aiello 1993, Wolpoff, Hawks *et al.* 2001, Caspari, Wolpoff 1996, and Wolpoff, Mannheim *et al.* 2004), although there were other intermediate models as well (Smith 1984, Smith, Janković *et al.* 2005, Bräuer 2008). In all models of modern human origins, there is an acknowledgement of an initial expansion out of Africa of *Homo erectus*, who settled in Europe and Asia between two and one million years ago (1-2 Ma; Aiello 1993). Very simplistically, Eurasian *H. erectus* is said to be the predecessor of the Eurasian Archaic hominins, such as *H. heidelbergensis*, who are further understood to be ancestral to Neanderthals. ("Archaics"

will be used to describe Middle and Late Pleistocene hominins which are not classified as Anatomically Modern Humans (AMHs), including those evolved from the earlier dispersal of *H. erectus* in Eurasia, such as the Denisovans and Neanderthals).

Chris Stringer, Leslie Aiello and colleagues (Stringer 2002, Stringer 2014, Aiello 1993) have laid out the four more-influential models of the late twentieth/early twenty-first century explaining the origin of modern humans:

1) *The Recent African Origins Model (RAO), or the African replacement model,* claims that modern humans evolved in Africa approximately 200 Ka and left Africa about 100 Ka. These colonisers replaced the populations existing in Europe and Asia with little to no hybridization. The RAO Model was developed by Chris Stringer, Peter Andrews and Ron Clarke in the mid-to-late 1980s, although it can trace its roots to Louis Leakey in the 1960s (Aiello 1993, Stringer, Andrews 1988, Clarke 1990).

2) *The (African) Hybridization and Replacement Model (HR)* championed by Bräuer (1984), included replacement by migrating modern humans from Africa, but allowed for some hybridization with the indigenous archaic populations.

3) *The Assimilation Model (AM)* also incorporated a recent African origin of all modern humans in its model, but emphasised gene flow and selection as important factors in the evolution of anatomically modern human (AMH) morphologies. This model, proposed by Fred Smith (Smith 1992, Smith, Janković *et al.* 2005), implies that certain morphological features seen in modern humans (e.g. in some parts of Eurasia), may be due to local continuity, brought about through gene flow.

4) *The Multiregional Model* of modern human origins argued that instead of recent African commonality, modern humans evolved both in Africa and in Eurasia, as the descendants of the earlier dispersal of *H. erectus*. This model emphasises that, through both regional genetic continuity, and gene flow between populations, modern humans evolved throughout the hominin-inhabited world. This model differs from C.S. Coon's model of early origins of geographically disparate "races" in that its proponents rejected the "candelabra" (polygenic) structure of human origins, where races evolved separately, and adopted instead a polycentric model, of a winding "multi-channelled stream, constantly dividing and merging" (Caspari, Wolpoff 1996, 265). Because of this, proponents of multiregionalism place their conceptual origin with Franz Weidenreich,

who labelled many of the archaic hominins, including East Asian *Homo erectus*, as potentially belonging to a single species of *Homo sapiens* (Weidenreich 1947).

While it may seem inconsequential to expand upon these ideas now that we have sequenced the genome of some Late Pleistocene hominins, understanding the structure of the debate is important for several reasons. First, the debate largely focussed on morphology (although support for the models using molecular evidences will also be briefly discussed), which was used to support both extremes (RAO and MR) of the argument, as well as the more intermediate models. It is wise to expand on some of these ideas to see which ones may be applicable to a model of human-Neanderthal hybridization, and which morphological features are less relevant. Second, while the extremes of the debate are no longer actively debated, the extent to which hybridization has contributed to the emergence of our species is still under discussion. Third, there is still some resistance to the intricacies of what constitutes genetic evidence for hybridization. These factors all stem from the initial ideologies and are important in creating a model of hominin hybridization. In this section, the evidence is presented (prior to 2010) for the two most polarising aspects of these debates: 1) modern humans evolved recently in Africa and migrating into Eurasia, replacing other hominin populations; and 2) hybridization could (and did) occur between divergent human populations. Evidence under these sections may support one (or more) of the models above.

Evidence for recent African origins of modern humans

Under the RAO, HR and AM models, modern humans originated in Africa around 100-200 Ka, dispersing and replacing other hominins throughout the world with some (HR; AM), if any (RAO), introgression (Stringer 2002, Stringer 1994, Stringer, Andrews 1988, Aiello 1993). Evidence for African origins initially focussed on the morphology of fossils. Louis Leakey suggested that Pleistocene remains in Africa (such as the "Chellean skull", a *Homo erectus* from Olduvai) seemed to have more human-like morphological features than East Asian *Homo erectus* (Leakey 1963). According to the RAO model, modernity included morphological features such as a high, rounded cranium, a mental eminence (chin) and a relatively gracile skeleton (Stringer 2002). Stringer and Andrews (1988) also added that modern humans were far less morphologically variable than the archaic populations in the early parts of the Late Pleistocene.

Proponents for this model highlighted the fact that the earliest appearance of anatomically modern humans (AMHs) in the hominin fossil record occurs in Africa. These include Klasies River and Florisbad in South Africa; Ngaloba in Tanzania; Herto, Aduma and Omo Kibish in Ethiopia; and

possibly Jebel Irhoud and Dar-es-Soltane in Morocco (dated between 80 and 260 Ka; Stringer, Andrews 1988, Aiello 1993, Singer, Wymer 1982, White, Asfaw *et al.* 2003, McDougall, Brown *et al.* 2005, Rightmire 1984, Hublin 1992, Reyes-Centeno 2016, Bräuer 2008). Furthermore, there seems to be morphological continuity between African specimens from the Middle Pleistocene into the Late Pleistocene (Reyes-Centeno 2016). Cranial transitional features between *Homo erectus* and *Homo sapiens* were seen in material from Bodo (Middle Awash, Ethiopia, c. 600 Ka), Elandsfontein (South Africa, 600 Ka to 1 Ma), Kabwe (Zambia, age comparable to Elandsfontein), possibly Salé (Morocco), Lake Eyasi (Tanzania) and Lake Ndutu (Tanzania; Rightmire 2009, Bräuer 2008). Some of the hominins listed as anatomically modern examples in Africa (see above) may fit more comfortably into this 'transitional' group and vice versa. The transitional features on late middle—late Pleistocene hominins found in Africa were thought of as a natural evolutionary transition, and not as evidence for gene flow between geographically disparate groups.

The Middle East or Levant, the most plausible corridor for an African exodus, yielded the earliest examples of anatomically modern humans outside of Africa (Valladas, Reyss *et al.* 1988). Within the Middle East, the earliest direct evidences for AMHs are from the Israeli sites, Qafzeh and Skhūl (120 to 80 Ka; Rightmire 2009, Mercier, Valladas *et al.* 1993). These specimens are rugged in appearance, with prominent supraorbital ridges, but share many anatomical features with modern humans (including, in many specimens, a chin, although this feature differs in morphology in these specimens compared with modern humans; Schwartz, Tattersall 2000). Indirect evidence for continuity of lithic technologies between sites in East Africa, particularly those associated with AMH, and those in the Arabian Peninsula between 128-74 Ka, were used to support the claim that these populations were migrants from Africa (Bar-Yosef 2002), although these claims were challenged (Fox, Coinman 2004). However, this occupation of the Levant by AMHs did not appear to be continuous, with apparent alternate occupations between AMHs from Africa and Neanderthals (such as Amud, dated to around 40 Ka) from Western Asia (Shea 2008). The intermediate features seen in early AMHs, RAO researchers insisted, and the early dates of fossils from Africa and the Levant, were starkly contrasted against specimens from Europe and Asia, which, they argued, did not show the same evidence for being transitional forms (Stringer 2002, Klein 1995).

Many researchers attributed the expansion and colonisation of Europe and Asia to a later exodus (or one of multiple "pulses") from Africa around 50 Ka and not to these earlier populations in the Levant (Klein 2000, Mellars 2006a, Stringer 2000, Shea, Sisk 2010), although this had been challenged (Armitage, Jasim *et al.* 2011). The earliest European AMHs (Cro-Magnon, Stetten and Mladeč) are younger than those seen in the Levant, supporting the RAO model (Stringer, Andrews 1988).

Furthermore, AMHs appearance in Europe was correlated with the disappearance of Neanderthals (Mellars 2006b), and attributed to changing environmental conditions (Finlayson 2004, Finlayson, Carrion 2007), or the technological superiority of modern humans (Mellars 2006a, Mellars 2006c). (This has also been attributed to the interbreeding and overwhelming migration of modern humans—Zilhão 2006—, which is more consistent with HR and AM).

Taxonomic "purity" of modern humans from Africa, compared with East Asian *Homo erectus* and European Neanderthals, was also supported by morphological cladistics, multivariate craniometric and dental analyses (Clarke 1990, Harvati 2003, Schillaci, Froehlich 2001, Turbón, Pérez-Pérez *et al.* 1997, Stringer, Humphrey *et al.* 1997, Stringer 1974, Stringer 1989, Bräuer, Rimbach 1990). These studies showed close and strongly overlapping morphological distances between modern human populations, when compared with Neanderthals and East Asian *H. erectus*. Modern humans (including Europeans) were statistically morphologically distant from Neanderthals, who exhibited relative lower cranial vaults, wider occipital regions and more projecting facial morphologies, which were enough to separate Neanderthals and humans in statistical analyses (Harvati 2003). Neanderthals were also morphologically distant from Late Pleistocene humans in Europe, the latter of which were shown to be more similar to modern humans (Turbón, Pérez-Pérez *et al.* 1997). Furthermore, early modern humans (Skhūl and Qafzeh specimens) more closely resembled Late Pleistocene humans than modern human groups (Bräuer, Rimbach 1990, Harvati 2003, Turbón, Pérez-Pérez *et al.* 1997). These lines of evidence were used to support the hypothesis that Neanderthals were taxonomically distinct, and that hybridization was unlikely (Harvati 2003, Harvati, Frost *et al.* 2005, Schillaci, Froehlich 2001, Harvati, Weaver 2006). Others highlighted that the more recent Neanderthal and East Asian *Homo erectus* specimens showed uniquely derived morphologies, more divergent from modern humans than earlier Eurasian hominins, to support speciation and diversification of these populations and render them less likely ancestors to modern populations than the less-derived African hominins (Clarke 1990, Stringer 1974). Even studies which showed some ambiguity as to the relationships between early AMHs, Neanderthals and Late Pleistocene hominins, did not propose unequivocal support for hybridization (Simmons, Falsetti *et al.* 1991).

Cladistic analyses based on dental non-metric trait variation were also interpreted as support of recent common origins among modern human groups, where Neanderthals were shown as dental outliers to all human geographic groups, including Upper Palaeolithic Europeans (Stringer, Humphrey *et al.* 1997, Bailey 2000, Bailey 2002b, Bailey 2002a). Furthermore, morphometric studies of tooth shape confirmed Neanderthal morphological distinctiveness (Bailey 2004, Bailey, Lynch 2005).

Ultimately, the most convincing argument supporting RAO arose from early molecular evidence. Mitochondrial, autosomal and protein molecular distances among human populations were shown to be smaller than that between many other species or subspecies studied (King, Wilson 1975, Ferris, Brown *et al.* 1981). Furthermore, the patterns of genetic variation (greatest genetic variation in Africa, larger intra-racial variation that interracial, etc.) in modern human populations supported a scenario of recent African human origins (Tishkoff, Dietzsch *et al.* 1996, Campbell, Tishkoff 2008, Quintana-Murci, Semino *et al.* 1999, Wainscoat, Hill *et al.* 1986, Greenberg, Newbold *et al.* 1983, Nei, Roychoudhury 1982). For example, less than 10% of protein polymorphisms differed between larger geographic "races", compared with the variation within them (Lewontin 1972, Latter 1980). Similarly, comparisons of mitochondrial DNA (mtDNA) across multiple human groups was consistent with an interpretation of recent common origins, with the famously dubbed "Mitochondrial Eve" calculated to have lived approximately 200 Ka (Cann, Stoneking *et al.* 1987, Vigilant, Stoneking *et al.* 1991, Horai, Hayasaka *et al.* 1995, Ingman, Kaessmann *et al.* 2000).

Correspondingly, a common ancestral Y-chromosome was calculated to be of a similar date, 188 Ka (Hammer 1995), and more recent studies calculated a more recent date (100 Ka; Ke, Su *et al.* 2001, Underhill, Passarino *et al.* 2001, Underhill, Shen *et al.* 2000, Thomson, Pritchard *et al.* 2000, Underhill, Kivisild 2007). The genetic evidence for low inter-population variation not only supported a RAO hypothesis, but inspired several sub-models in order to explain the geographic distribution of genetic (mainly mtDNA) variability: Replacement, Weak Garden of Eden and Multiple Dispersals (Ambrose 1998, Harpending, Sherry *et al.* 1993). These models varied in the extent to which they attributed human variation in genetic structure to bottlenecks, genetic drift and/or sudden population expansion; none considered gene flow as a dominant force.

Successes in retrieving ancient mtDNA from Neanderthals in the 1990s yielded greater supporting evidence for little to no hybridization (Serre, Langaney *et al.* 2004, Krings, Stone *et al.* 1997, Krings, Geisert *et al.* 1999, Ovchinnikov, Götherström *et al.* 2000, Schmitz, Serre *et al.* 2002, Orlando, Darlu *et al.* 2006, Caramelli, Lalueza-Fox *et al.* 2006, Lalueza-Fox, Sampietro *et al.* 2005). Neanderthals were determined to be a distinct out-group from modern human populations, with divergence estimated around 465 Ka. Furthermore, Neanderthals did not appear to contribute mtDNA to early modern humans in Europe (Serre, Langaney *et al.* 2004). These studies supported RAO, and their results were subsequently reinforced by the sequencing of partial Neanderthal genomic DNA (Noonan, Coop *et al.* 2006).

Methodological Criticisms of Recent African Origins

While many lines of evidence appeared to support RAO, many of these were criticised (or at least did not eliminate the possibility of hybridization). For instance, mtDNA and Y chromosomal DNA of modern human populations can only tell us about the maternal and paternal lineages of modern people, and need not be ancestral to any other sequences of DNA (Jobling, Tyler-Smith 1995). Furthermore, selection has been shown to heavily influence mtDNA evolution and the evolution of sex chromosomes which may have affected the heritability of these sequences (Gillespie 2001, Nachman, Brown *et al.* 1996, Merriwether, Clark *et al.* 1991). This means that Neanderthal-derived DNA could still account for a large proportion of the genome of contemporary modern human individuals or groups. Therefore, lack of contribution of Neanderthal mtDNA to modern populations did not exclude the possibility of hybridization. Furthermore, selection can account for the survival of recently-derived mtDNA and Y chromosomal DNA in modern humans.

The extent to which Neanderthals could have interbred with modern humans without leaving mtDNA in contemporary populations was modelled in order to test for the potential extent to which hybridization could have taken place among these groups. One model simulating demographic scenarios of admixture between Neanderthals and migrating AMHs indicated the possibility of introgression to be as high as 25% (Serre, Langaney *et al.* 2004). However, other models calculated introgression as less than 0.2% or even 0.1% (Currat, Excoffier 2004, Weaver, Roseman 2005). These were highly dependent on the parameters used within the models. Regardless of the extent to which hybridization took place, it was argued that even a small amount of gene flow could result in adaptive introgression, which could have profound effects (Hawks, Cochran 2006, Hawks, Cochran *et al.* 2008).

Studies supporting inter-specific relationships between Neanderthals and humans using morphometrics were also criticised for using techniques which may inflate inter-group variability (Ackermann 2005, Wolpoff, Mannheim *et al.* 2004). Some have also suggested that the trends towards anatomical modernity (e.g. increased gracility) from early modern humans can also be seen in Neanderthals. Neanderthals have, themselves, tended towards an increasingly "modern" morphology in the Late Pleistocene in Western Europe, potentially due to early introgression, as was argued for Amud (Wolpoff, Mannheim *et al.* 2004), St Césaire (Trinkaus, Churchill *et al.* 1999) and even Vindija (Smith 1984, Wolpoff 1999). (The ancient genomes of Neanderthals such as Vindija have since not supported this scenario for those individuals; Green, Krause *et al.* 2010). However, while many argued that the tool technology seen in the Levant could be traced to Africa; there was

also evidence that technologies were shared between Neanderthals and modern humans (Frayer 1986, Mercier, Valladas *et al.* 1993).

Evidence for Hybridization in the Hominin Fossil Record (Support for HR and AM)

Although RAO has been the dominant narrative of human origins, there has also been evidence brought forward that supports the alternative hypotheses. This includes evidence for hybridization, as it shows that multiple lineages contributed to human origins, and not only African. The Multiregionalism model initially stressed continuity from Middle to Late Pleistocene, with a small amount of gene flow keeping populations connected and evolving together. However, hybridization became a more fundamental part of the model, and more greatly emphasised by proponents, during the early 2000s.

The complex populating of the Levant (inhabitation by early modern humans, followed by their replacement by Neanderthals and the subsequent re-inhabiting of the Levant by another "pulse" of modern humans) has also been questioned. Some argue that this is evidence for sympatry, and that most specimens in the Levant between 100-50 Ka were not convincingly assigned to either AMHs or to Neanderthals (Wolpoff, Mannheim *et al.* 2004). Hominins which were argued to display both human and Neanderthal traits have long been suggested as potential hybrids, or members of introgressed populations. Individuals from Skhūl (Israel, 100 Ka) have mainly modern human features with certain Neanderthal traits: chinless or with a retromolar space (Wolpoff, Mannheim *et al.* 2004).

Within Europe, there were also contentious specimens with apparent mixed modern-Neanderthal morphologies in the mid-to-late Late Pleistocene, casting doubt on the validity of a clear "replacement" of Neanderthals by AMHs (Wolpoff, Hawks *et al.* 2001, Wolpoff, Caspari 2011, Wolpoff, Mannheim *et al.* 2004, Trinkaus 2007). Hominin fossils from Mladeč Caves (Czech Republic, dated to 31 Ka) were central to some of the discussions around modernity (Wild, Teschler-Nicola *et al.* 2005, Wolpoff, Hawks *et al.* 2001). They were dated to within the time-frame of transition and, while undisputedly representative of modern humans, shared traits some argued as having inherited from Neanderthals (Trinkaus 2007). These included occipital bunning, large dentition, and robust postcrania and supraorbital regions (Wolpoff, Hawks *et al.* 2001, Frayer 1986, Trinkaus 2007). Furthermore, there was a high degree of morphological variability among these specimens (Wolpoff, Caspari 2011, Smith 2013). Similarly, human remains from Peştera Muierii (Romania, 30 Ka) were argued to represent AMHs, based on numerous modern human traits. Others have suggested that

certain features were more similar to those seen in Neanderthals, including a large occipital bun and postcranial morphologies (Soficaru, Dobos *et al.* 2006). The appearance of Neanderthal traits in modern humans in Europe occurs in a patchwork manner, possibly indicating variability in Neanderthal-human reticulation, with some of these traits observed in even more recent Europeans, such as Cro-Magnon (Trinkaus 2007, Smith 2013).

The Lapedo child (from Largo Velho, Portugal dated to around 24.5 Ka) seemed to show a mix of Neanderthal and modern human features (Zilhão 2008, Zilhão 2001, Duarte, Mauricio *et al.* 1999). While Neanderthals had largely been replaced in Eastern Europe before 30 ka, parts of Western Europe (including Iberian regions) were possibly only occupied by modern humans as recently as 28 Ka (Zilhão 2001). However, since there is no associated recent Mousterian culture, no other examples of individuals with mixed morphologies had been found in the region, and the child lived well after hybridization could have initially taken place, others stressed that it was more likely that the specimen was "simply a chunky Gravettian child" (Tattersall, Schwartz 1999, 7119). Furthermore, some of the Neanderthal traits (such as the occipital bun) were suggested as being within the range of Late Pleistocene human variation (Arsuaga, Villaverde *et al.* 2002).

It is important to be aware that hybridization among human groups in the Late Pleistocene was not confined to evidences involved in human and Neanderthal genetic exchange. Using data on human genetic variation from the National Institute of Environmental Health Sciences, researchers supported the hypothesis that gene flow from archaic hominins could explain at least 5% of loci genetic variants in non-Africans and in West Africans (Wall, Lohmueller *et al.* 2009, Plagnol, Wall 2006).

The origins of modern humans in Asia and Australia were also scrutinized. The Willandra Lakes Hominid (WLH) 50, a cranium from Australia dated around 13-15 Ka, is argued to show morphological elements that closely resemble early Indonesian fossil hominins (Wolpoff, Hawks *et al.* 2001, Hawks, Oh *et al.* 2000). Indeed, they demonstrated that there were fewer morphological differences between WHL50 and Ndagong specimens from Indonesia (late *H. erectus*/early archaic humans) than from early modern humans such as Omo, Jebel Irhoud, Skhūl and Qafzeh. DNA from AMH remains from Australia showed that, while later AMHs in Australia have the fixed mtDNA lineage shared by all modern humans, Mungo 3 (dated to 60 Ka) had mtDNA belonging to a more divergent lineage (Adcock, Dennis *et al.* 2001). This has since been shown to be the result of contamination (Heupink, Subramanian *et al.* 2016).

Besides the fossils, there was other support for physical interaction (if no hybridization) between AMHs and archaics. Genetic analyses of head lice have shown that there are two lineages which diverged around 1.18 Ma. One of these lineages underwent a bottleneck around 100 Ka, mimicking the bottlenecking of modern humans dated to a similar time-period. The other lineage is found in the Americas (Reed, Smith *et al.* 2004). Such a scenario strongly suggests that the latter lineage of the parasite switched hosts as ancestors of Native Americans spread through Asia and eventually into the Americas. This supports direct contact between modern humans and other archaic populations in Asia.

Animal models for hominin hybridization

As support for both the Multiregional Model and Recent African Origins dwindled, models which incorporated the importance of hybridization as a common theme were still argued to best represent what is seen in other species. Many researchers highlighted the fact that animals, particularly primates, of similar or greater genetic divergence, hybridize, or show high levels of past introgression (Ackermann 2010, Jolly 2001, Arnold, Martin 2009, Arnold 1992, Arnold, Meyer 2006, Arnold 2008). It seemed unlikely that, as part of an order of mammals with such abundant hybridization, and with high levels of taxonomic diversity, late Pleistocene hominins would not hybridize with one another. Furthermore, as discussed above, evidence supporting RAO was either contentious, or did not completely rule out hybridization and gene flow.

Animal hybrids have shown that mosaic features are not the only potential indication of hybridization in the morphology (as argued in Chapter 3). High levels of unusual or rare traits of late Pleistocene hominin specimens were seen as evidence for hybridization. Some non-metric traits, rare in pure or parental populations, have been shown to be more common in hybrids and recombinants (Ackermann, Schroeder *et al.* 2014, Ackermann, Brink *et al.* 2010, Ackermann, Rogers *et al.* 2006, Ackermann, Bishop 2010, Fuzessy, de Oliveira Silva *et al.* 2014, Ackermann 2010). If hybridization were, indeed, rare it seemed unlikely that these traits would show up as part of the hominin fossil record at all. Ackermann (2010) had listed numerous potential hominin candidates for hybridization based on unusual trait morphologies, including Peştera cu Oase 2. The Romanian cranium, dated to 35 Ka (revised to 37-42 Ka, Fu et al. 2015), had molars that were extremely large compared with both Late Pleistocene modern humans and Neanderthals (Trinkaus, Moldovan *et al.* 2003, Rougier, Milota *et al.* 2007, Trinkaus 2007). Ackermann argued that such unusual features, pointed out in the original paper as possible evidence for introgression, are certainly consistent with hybridization. (In 2015, ancient DNA analyses concluded that Oase 1, an associated mandible from a

different individual which shared similarly unusually large molars, was a 6th to 8th generation Neanderthal-human recombinant; Fu, Hajdinjak *et al.* 2015).

Ackermann (2010) also listed other potential late-Middle and Late Pleistocene hybrid candidates. The Krapina hominins, dated to 130 Ka, show unusually high levels of premolar rotation. Considering there is little evidence for modern humans in Europe at that time, human-Neanderthal hybridization was seen as an unlikely contributor to these anomalies. However, since then, it has been shown that gene flow between humans and Neanderthals had been ongoing for tens of thousands of years before the first humans are documented in Europe (Fu, Hajdinjak *et al.* 2015, Fu, Li *et al.* 2014). Furthermore, Ackermann argued that it is also likely that introgression occurred between multiple, yet unknown archaic hominins, possibly explaining some of these features. Since then, such a scenario of introgression among multiple hominins has been supported by both the ancient DNA and interrogation of the human genome (Reich, Green *et al.* 2010, Prüfer, Racimo *et al.* 2014, Hammer, Woerner *et al.* 2011).

Ackermann (2010) also noted unusual morphologies in hominin specimens from the Levant, such as a rotated premolar in Skhūl IV, and high levels of craniofacial asymmetry in Skhūl V. Qafzeh (Israel, 95 Ka) specimens are also considered anatomically modern, but with a high degree of morphological variability, and dental crowding in several individuals (and a rotated premolar in Qafzeh 11). These features are consistent with developmental instability, which can arise in hybrids due to the combining of divergent genomes (Ackermann 2007). These specimens (Skhūl and Qafzeh) are also associated with Mousterian technology (which is considered Neanderthal in origin). These may also be indicators of a more complex scenario of interaction, both culturally and biologically. Similarly, Amud 1 (Israel, 50 Ka, assigned as Neanderthal) shows incredibly high cranial heterosis (large size) and a reduced maxillary molar (Ackermann 2010). These affects in animal hybrids are argued to be due to developmental breakdown (Ackermann 2007), or to complementary gene action (see chapter 3).

Another potential candidate for hybridization between anatomically modern humans and archaics may be LB1, the type specimen of *Homo floresiensis*. While others have suggested that the small-bodied, small-brained hominin morphology may either be due to pathology (largely disregarded) or insular dwarfism (Baab, McNulty 2009, Jacob, Indriati *et al.* 2006, Henneberg, Thorne 2004, Brown, Sutikna *et al.* 2004, Falk, Hildebolt *et al.* 2009a, Falk, Hildebolt *et al.* 2009b), Ackermann (2010) points out that bilaterally rotated premolars, as seen in this specimen, are consistent with hybrid morphologies (as seen in wildebeest hybrids). While a small-bodied potentially ancestral population of *Homo floresiensis* has since been found (Brumm, van den Bergh, Gerrit D *et al.* 2016, van den

Bergh, Gerrit D, Kaifu *et al.* 2016), supporting the argument for insular dwarfism, it does not negate *H. floresiensis* as a potential taxon with which other late Pleistocene hominins may reproduce. The refined date of *H. floresiensis* also places these specimens in a more likely timeframe of modern human expansion (Sutikna, Tocheri *et al.* 2016).

Post-2010 Evidence for Late Pleistocene Hominin Introgression

Recently, views of modern human origins have become more balanced. The ancient genomic evidence seems to appropriately explain the contradictions inherent in both the fossil and modern genetic evidence. While a recent expansion of AMHs from Africa is well-corroborated, so too is evidence of fairly extensive introgression between these expanding populations and archaic hominins both in Eurasia and in Africa. Current evidence (genetic and morphological) now supports a model of human origins that more closely resembles the (African) Hybridization and Replacement Model or the Assimilation Model, where both African origins and archaic gene flow is accounted for to a greater or lesser extent. Ackermann and colleagues (2016) have further extended these models to include hybridization not only as a factor within human evolution, but also as a creative force driving the variability and adaptability which characterises our lineage (Ackermann, Mackay *et al.* 2016).

Neanderthal Ancient DNA

Since the sequencing of Neanderthal autosomal DNA, our understanding of the intricacies of hybridization among late Pleistocene hominins boomed. Green et al (2010) presented a draft sequence of the Neanderthal genome (over 4 billion nucleotides) from three female Neanderthals from Vindja Cave (Croatia), dated to around 40 Ka, and smaller DNA sequences with Neanderthals from El Sidron (Spain), Feldhofer Cave (Germany), and Mezmaiskaya Cave (Russia), all dated to the Late Pleistocene (Green, Krause *et al.* 2010). In this analysis, the researchers estimated a divergence date between humans and Neanderthals of approximately 800 Ka (population splitting completely at 300 Ka). They also remarked that, although the Neanderthals sequenced are from a great geographic and temporal range, they have similar genomes, and are all comparably distant to modern humans living today. Analysis of the genome also yielded evidence for positive selection in

modern humans, with genes associated with cognition, skeletal development and metabolism differing from those of the Neanderthals (Green, Krause *et al.* 2010).

Most importantly for this study, Neanderthals were shown to share more genetic variants with populations outside of sub-Saharan Africa than within, providing substantial evidence for interbreeding between them and humans that had migrated out of Africa (Green, Krause *et al.* 2010). Shared genetic variants of non-sub-Saharan Africans and Neanderthals ranged between 1-4% (often now revised to 1-3%; Vernot, Akey 2014). Interestingly, however, Neanderthals were not more closely related to Europeans than to East Asians. Furthermore, around 20% of the Neanderthal genome has been recovered in modern populations, despite each individual having approximately only 2% Neanderthal DNA (Vernot, Akey 2014, Sankararaman, Mallick *et al.* 2014).

Following this initial study, a number of studies offered possible alternative explanations for the observed pattern. For example, it was suggested that ancestral populations that left Africa around 50 Ka might have been more similar to Neanderthals than to populations that left within Africa due to ancient substructure (Green, Krause *et al.* 2010, Eriksson, Manica 2012). However, this was not supported by some models of the genome (Yang, Malaspinas *et al.* 2012), and studies measuring linkage disequilibrium in modern European populations confirmed the gene flow hypothesis, dating interbreeding to between 37-86 Ka (Sankararaman, Patterson *et al.* 2012). Yet other models further emphasized that the calculated genetic similarity overemphasizes hybridization when spatial expansion isn't taken into account, and could be less than 2% (Currat, Excoffier 2011). Such low rates, argue the authors, imply that hybridization was extremely rare, further implying difficulties often seen in inter-specific hybridization. Still others showed that hybridization only need occur once every 77 generations in order for Neanderthal genes to be detected in modern non-Africans at the rate of 1-4% (Neves, Serva 2012).

Subsequent genomic analyses of early modern humans in Eurasia supported scenarios of more extensive gene flow between Neanderthals and modern humans. A modern human near Ust'-Ishim (western Siberia, dated to approximately 45 Ka), from a population living before or around the time of eastern/western Eurasian population separation, showed similar proportions of Neanderthal ancestry as seen in living Eurasian populations (around 2.3%; Fu, Li *et al.* 2014). The size of the introgressed segments in the Siberian specimen, however, were longer, indicating more recent Neanderthal ancestry than that of contemporary modern Eurasians, at approximately 7-13 Ka before the individual lived (approximately 50-60 Ka) and possibly a small proportion of more recent admixture (Fu, Li *et al.* 2014). Yet another modern human specimen from Russia (Kostenki 14, around 38 Ka) shares close ancestry with European Mesolithic hunter-gatherers and modern day

Europeans, thus post-dating the east-western Eurasian separation, and contains more Neanderthal DNA of longer tracts (Seguin-Orlando, Korneliussen *et al.* 2014). And an early modern human from Tianyuan Cave (China, from approximately 40Ka), that lived after the separation of western and eastern Eurasian populations and was derived from a population ancestral to Native Americans and Asians, showed similar proportions of Neanderthal DNA as that seen in modern populations (Fu, Meyer *et al.* 2013). Gene flow thus had already occurred before these individuals were alive, and it is possible that selection had already acted on introgressed sections of the genome.

A modern human from Peştera cu Oase (Oase 1 mandible, from Romania, dated to around 40 Ka) was also analysed using ancient DNA techniques, and presented with 6-9% Neanderthal-derived DNA, indicating a Neanderthal ancestor of 4-6 generations prior (Fu, Hajdinjak *et al.* 2015). This individual, and the cranium of another associated individual, Oase 2, were previously suggested to have features consistent with Neanderthals or with hybrids more broadly (Ackermann 2010, Rougier, Milota *et al.* 2007, Trinkaus, Moldovan *et al.* 2003). Oase 1 was not, however, more closely related with western than eastern Eurasians, indicating that these early modern human populations which interbred with Neanderthals did not necessarily contribute significantly to contemporary European populations (Fu, Hajdinjak *et al.* 2015).

The Middle East has been viewed as an ideal region to search for evidence of hybridization between modern humans and Neanderthals (Sankararaman, Patterson *et al.* 2012). This is especially so when one considers that Neanderthals contributed similarly to both East Asians and Europeans (or even more greatly to modern East Asians), despite the assumed extensive sympatry of the two groups in Europe (Wall, Yang *et al.* 2013, Meyer, Kircher *et al.* 2012). Thus this was seen as the likely geographic space wherein hybridization and introgression most intensively had taken place. However, the evidence from Ust'-Ishim and Peştera cu Oase support a more complex series of hybridization events, which could have occurred throughout Europe and Asia, and over a fairly long time (Fu, Li *et al.* 2014, Fu, Hajdinjak *et al.* 2015). Furthermore, some gene flow between the taxa seems to have occurred after the separation of eastern and western Europeans (Wall, Yang *et al.* 2013, Vernot, Akey 2015). It is also worth noting that migration and interbreeding among more recent humans (and presumably archaics) makes this pattern more complex. Wall et al (2013), for instance, found a small proportion of Neanderthal DNA in the Maasai in East Africa (Wall, Yang *et al.* 2013).

THE HIDDEN HOMININS: DENISOVANS AND OTHERS

One of the more astounding results of these recent ancient DNA studies has been the emerging genetic evidence for previously unknown hominin taxa. The first such revelation was based on the DNA from a finger bone in Denisovan Cave (Siberia, dated to at least 50 Ka), and corroborated with the mitochondrial genome from a very large tooth (Reich, Green *et al.* 2010, Krause, Fu *et al.* 2010). The mtDNA of the Denisovans appears to be "exceptionally archaic" (Reich, Green *et al.* 2010, 1059), showing some discordance with the autosomal DNA, which suggests a more recent share lineage with Neanderthals. These "Denisovans" shared a more recent common ancestor with Neanderthals than modern humans, yet underwent a divergence from Neanderthals as far back as 640 Ka (Reich, Green *et al.* 2010), not long after the split with modern humans (approximately 800 Ka). Others have refined this date to be later for both the modern-archaic split (570 Ka) and the Neanderthal-Denisovan split (380K a; Prüfer, Racimo *et al.* 2014). Furthermore, the morphology of the tooth (maxillary molar) shares none of the derived features of either Neanderthals or modern humans. What is more astounding is that less than 100km from the Denisovan Cave, Neanderthal DNA had been extracted from specimens in Okladnikov Cave at a similar time-range (Reich, Green *et al.* 2010).

Unlike the Neanderthals, the Denisovans did not contribute genetic material to all Eurasians, although it is likely that gene flow between Denisovans and Neanderthals occurred before Neanderthals interbred with modern humans (Reich, Green *et al.* 2010). However, there is evidence for gene flow from Denisovans into some Asian populations. Melanesians appear to have derived 4-6% (some populations 2-4%; Vernot, Tucci *et al.* 2016) of their genome from Denisovans; this DNA is also found in Australian Aborigines and other Southeast Asian populations (Reich, Patterson *et al.* 2011, Reich, Green *et al.* 2010). Some have also highlighted modern genomes from mainland Asia and Native America which have low levels of DNA of Denisovan ancestry (approx. 0.2%; Skoglund, Jakobsson 2011, Prüfer, Racimo *et al.* 2014, Qin, Stoneking 2015), although this may be due to later gene flow among modern human groups. Late Pleistocene modern humans from Tianyuang Cave (China) and Ust' Ishim (Russia) also showed no evidence for Denisovan introgression (Fu, Meyer *et al.* 2013, Fu, Li *et al.* 2014), but this may be due to there being low levels of introgression in mainland Asia even in the deeper past (Prüfer, Racimo *et al.* 2014). Another option is that multiple dispersals into Asia may have diluted the Denisovan signature in mainland Asia and the Americas (Reyes-Centeno, Ghirotto *et al.* 2014, Reyes-Centeno 2016). Because much of the introgressed Denisovan genome is found in populations in Southeast Asia, the Denisovans were possibly incredibly widespread, occupying much of Asia (Reich, Patterson *et al.* 2011).

A proximal toe phalanx was later found in the Denisovan Cave, in a lower layer than the finger phalanx discussed above, genetically forming a clade with Neanderthals (Prüfer, Racimo *et al.* 2014).

Signals of both Neanderthal gene flow and that of an unknown archaic hominin into Denisovans have also been found (Prüfer, Racimo *et al.* 2014). The unknown archaic hominin appears to be more greatly divergent than the Neanderthal-Denisovan-modern-human cluster (diverged over 1 Ma), and has contributed around 0.5-8% of the Denisovan genome, although this may also be explained by complex population structures (Prüfer, Racimo *et al.* 2014). This, along with the archaic nature of the Denisovan mtDNA, possibly supports introgression into the Denisovan lineage from a more divergent hominin. Biological interaction among archaics was further supported by mtDNA extracted from Sima de los Huesos: a middle Pleistocene assemblage with hominins displaying numerous Neanderthal-like morphological characteristics yet assigned to *Homo heidelbergensis*. The mitochondrial genome, however, was more closely related to the Denisovans (Meyer, Fu *et al.* 2014).

Since the sequencing of the Neanderthal and Denisovan genomes, hominin finds in East Asia have been looked at with regards to an increasingly complex history of modern human origins. Specimens in South China, dated to the beginning of the Late Pleistocene, have multiple morphological affinities to modern humans, suggesting early modern human occupation of eastern Asia (Curnoe, Ji *et al.* 2012, Curnoe, Ji *et al.* 2015). Teeth from Huanglong Cave and Luna Cave (Southern China) were dated to the early Late Pleistocene, and fall within the range of modern populations in China (Liu, Wu *et al.* 2010, Shen, Wu *et al.* 2013, Bae, Wang *et al.* 2014, Curnoe, Ji *et al.* 2015). The Xujiayao specimens (North China) have been argued to show mixed morphologies in the mandibular ramus between more modern features and those seen in archaics and Neanderthals (Wu, Trinkaus 2014), temporal labyrinths that more resembles the derived Neanderthal morphology (Wu, Crevecoeur *et al.* 2014), and dentition which showcase a mosaic of primitive and derived features (Xing, Martinón-Torres *et al.* 2015). These complex features may point to a surviving hominin lineage in eastern Asia, or to gene flow and interaction among Middle-to-late Pleistocene hominins (Wu, Trinkaus 2014).

Similarly, two crania from Xuchang, China, dated to 105-125 Ka, exhibit mixed morphologies: eastern Eurasian Middle Pleistocene archaic cranial shape and incredibly large brain size, with Neanderthal-like occipital and temporal morphologies (Li *et al.* 2017). These were argued to reflect regional continuity into the Late Pleistocene in eastern Asia, as well as support east-to-west gene flow.

Understanding hybridization on the African continent has been more problematic. There are no pre-Holocene ancient genomes that have been sequenced from African populations. (One ancient Holocene Ethiopian genome was sequenced, indicating western Eurasian backflow into East Africans; Gallego Llorente, Jones *et al.* 2015. However, the original claim of extensive genetic backflow into sub-Saharan Africans, however, was overestimated). This may be due to conditions

which are more hostile to the preservation of genetic material, yet this region of study is likely to be the "next frontier" of ancient genomics. Some studies have used DNA sequences of contemporary modern human populations to model the likelihood of recent introgression from archaic populations to modern humans within Africa. In one study, approximately 2% of the genome of sub-Saharan African populations appeared to have introgressed around 35 Ka from an archaic population that diverged approximately 700 Ka (Hammer, Woerner *et al.* 2011). Similarly, when studying African hunter-gatherer populations (Pygmies from Cameroon and Hadza from Tanzania and the San), one study indicated evidence for archaic introgression into all three (Lachance, Vernot *et al.* 2012).

ADAPTIVE INTROGRESSION

While hybridization is important for generating morphological, physiological and genetic variability, selection has also been shown to have a role in maintaining or retiring introgressed genes and features (Hedrick 2013). Adaptive introgression, as evidenced by the retention of potentially adaptive genes in one population from another, allowed for better survival of hominins expanding into new, foreign environments. This is important because acquiring biological variation through introgression is considerably faster than acquisition through mutation alone (Grant, Grant 1994). Hominins already living in these landscapes for hundreds of thousands of years would have evolved the appropriate adaptations necessary to thrive in these environments; acquiring these adaptations through hybridization is an effective means for rapid success.

Some haplotypes involved in skin morphology and physiology have introgressed into certain living human populations from Neanderthals. BNC2, involved in skin pigmentation, occurs at levels of 70% in Europeans, yet is not present in Asians (Vernot, Akey 2015, Sankararaman, Mallick *et al.* 2014, Racimo, Marnetto *et al.* 2016). Conversely, the Neanderthal haplotype causing loss-of-function in the melanocyte-stimulating hormone receptor gene (MC1R), involved in skin colour, is incredibly high in Taiwanese populations (~65%) and East Asians (~30%), but not in Europeans (~5%; Ding, Hu *et al.* 2014a). High frequencies of Neanderthal-derived alleles affecting keratin formation have also been found (Sankararaman, Mallick *et al.* 2014). POU2F3, involved in epidermal barrier function through keratinocyte differentiation, is found in 66% of East Asians, but is almost non-existent in Europeans. Furthermore, one chromosomal region (12q13), introgressed into both East Asians and Europeans, contains clusters of genes involved in keratin manufacture (Vernot, Akey 2015). Another chromosomal region (3p21.31), with genes involved in responses in ultraviolet-B (such as HYAL2), has over 50% presence in East Asians (variable, yet correlated with latitude) and is low in Europeans

(Ding, Hu *et al.* 2014b). Therefore, the evolution of skin colour and function in humans includes selection for introgressed gene variants.

Haplotypes inherited from Denisovans have also been shown to be adaptive in modern human populations. A haplotype of EPAS1 has been highly selected for in Tibetans at high altitudes. In environments where oxygen-deprivation often leads to increased haemoglobin concentrations in the blood (a risk-factor for preeclampsia in pregnancy), the EPAS1 variant has been shown to allow Tibetans to thrive by thresholding these concentrations. Furthermore, this variant is closely related to that of Denisovans, and is one of the strongest genetic candidates for adaptive introgression (Huerta-Sánchez, Jin *et al.* 2014). Similarly, haplotypes of genes involved in body fat distribution (WARS2 and TBX15) in native Greenland populations appear to be from an archaic population related to the Denisovans (Racimo, Gokhman *et al.* 2017).

Genetic variants involved in immune function and lipid metabolism in living people have also introgressed from archaics (Mendez, Watkins *et al.* 2012b, Racimo, Marnetto *et al.* 2016). Among the genes with the highest proportions of Neanderthal ancestry, many are involved in immunity (Dannemann, Andrés *et al.* 2015). These include variation in STAT2 and a cluster of OAS immunity genes, although frequencies of the latter in modern populations may be the result of neutral evolution (Mendez, Watkins *et al.* 2013, Mendez, Watkins *et al.* 2012a). The STAT2 variant, however, is seen in far higher frequencies in Melanesians than other Eurasian populations, possibly supporting a scenario of more complex hybridization and gene flow among archaics in Eurasia before modern human expansion (Mendez, Watkins *et al.* 2012a). Other candidates for adaptive introgression from both Denisovans and Neanderthals are the HLA class 1 (important for immune detection) and toll-like receptor (innate immunity) gene variants, which have been shown to be present in high proportions in Eurasian populations (Abi-Rached, Jobin *et al.* 2011). High frequencies of the OAS1 Denisovan-derived hyplotype show potential introgressive immune adaptation (Mendez, Watkins *et al.* 2012b).

Genes involved in immunity will be under high selective pressures, and introgression may have allowed for greater survivability of encroaching populations into new environments with a different set of evolved pathogens. Furthermore, adaptive introgression of immune-functioning genetic haplotypes may not be limited to exchanges between modern humans and the archaics. Prüfer and colleagues (2014) showed that some genes involved in immunity (HLA and CRISP cluster), may have introgressed from Neanderthals into Denisovans, with these haplotypes sharing more recent common ancestry than other parts of the genome (Prüfer, Racimo *et al.* 2014).

Interestingly, Neanderthal alleles found at high frequencies in some human populations have also been associated with schizophrenia, hydrocephaly, skin and lacrimal gland cancer, diabetes (I and II) and muscular dystrophy (Racimo, Marnetto *et al.* 2016). Neanderthal-derived alleles have also been implicated in lupus, smoking addiction and Crohn's disease in living Eurasians (Sankararaman, Mallick *et al.* 2014). It is unknown how these now-deleterious gene variants survived into modern populations, but it may be due to the fact that these genes became deleterious only in conjunction with a modern western lifestyle, and may in fact have neutral or positive functionality in the past (Ackermann, Mackay *et al.* 2016).

Adaptation against introgressed genes has also occurred. Over the last 45 Ka, the proportion of Neanderthal DNA retained in modern humans has decreased slightly, from between 3-6% to modern levels (Fu, Posth *et al.* 2016). Since most individuals analysed stem from an initial small founding population (that does not appear ancestral to modern Europeans), admixture cannot explain the bulk of this trend. Thus, it appears to support a scenario of selection against some Neanderthal alleles (Fu, Posth *et al.* 2016), although low population density of Neanderthals may also explain this trend (Smith, Lacy *et al.* 2015, Churchill 2014, Juric, Aeschbacher *et al.* 2015). This is further supported by the fact that the proposed importance of genes for function appears negatively correlated with the proportions of the Neanderthal alleles in modern populations (Sankararaman, Mallick *et al.* 2014, Vattathil, Akey 2015), and the finding of large regions of the genome (particularly on chromosome 7) strongly depleted of Neanderthal variants (Vernot, Akey 2015). More specifically, this depletion is seen on the X-chromosome, where Neanderthal ancestry in Eurasians is far smaller than in other chromosomes, and in genes which are mainly expressed in the testes (Sankararaman, Mallick *et al.* 2014). Furthermore, this pattern of lower introgression as seen on the X-chromosome, is also noted in native Papuans with respect to Denisovan ancestry (Meyer, Kircher *et al.* 2012). Multiple genes on the X-chromosome have been implicated in hybrid male sterility in other animals, potentially providing evidence for Haldane's Rule acting within late Pleistocene hybridizing hominins (Sankararaman, Mallick *et al.* 2014, White, Stubbings *et al.* 2012). Some studies have also shown that selection against Neanderthal genomic variants may explain the reduced Neanderthal material in early late Pleistocene modern humans, which may have initially led to less-fit hybrid offspring (Harris, Nielsen 2016, Juric, Aeschbacher *et al.* 2015).

A NOTE ON BEHAVIOURAL "MODERNITY"

Many researchers include in the narrative of expansion of modern humans out of Africa evidence for behavioural modernity, such as the use of symbolism (and possibly language), blade tools and

projectile weapons (Stringer 2002, Klein 2000, Klein 2008, Shea, Sisk 2010, Mellars 2006c, Mellars 2004). The presence of archaeological evidences of symbolism and technologies in Africa from as early as 70 Ka, which include the engraved ochre at Blombos, perforated shells and bone tools, has been used as support for RAO because it is earlier, say supporters, than that recorded in Eurasia (Henshilwood, D'errico *et al.* 2001, Henshilwood, d'Errico *et al.* 2002, Henshilwood, d'Errico *et al.* 2009, Henshilwood 2007, Henshilwood, d'Errico *et al.* 2004, McBrearty, Brooks 2000, Marean, Bar-Matthews *et al.* 2007, d'Errico, Henshilwood 2007, d'Errico, Henshilwood *et al.* 2005). In this scenario, the replacement of Neanderthals in Western Asia and Europe was associated with the spread of the well-documented Aurignacian cultural material between 35-40 Ka; this material was said to be associated with anatomically modern humans at sites such as Peştera cu Oase (Romania), Le Rois (France), Ksar Akil (Lebanon) and Mladeč (Mellars 2004). The timing and nature of this replacement was seen as evidence for the evolution of a "package" of behavioural and anatomical modernity, evolving in Africa and ultimately replacing Neanderthals and other Archaics in Eurasia (i.e. RAO).

This is not without contention. Opponents have criticized the "package" of behavioural and anatomical modernity by listing evidences of European, Australian and East Asian symbolism pre-Aurignacian, or pre-AMH expansion (Wolpoff, Mannheim *et al.* 2004, Zilhão 2006). Such evidences include the Shanidar flower burials, associated with Neanderthals (Leroi-Gourhan 1975, Solecki 1975), although the evidence presented was questioned.

Stronger arguments against the "package" of anatomical and behavioural modernity lay in the apparent disjunction, or time lag, between the appearance of modern humans and the material argued to represent behavioural modernity (McBrearty, Brooks 2000). In their argument, McBrearty and Brooks point out that the tool technologies (bone and microlithic), art, long distance trade and other features occur gradually over space and time: from between 300-50 Ka. Thus, the archaeological record may be interpreted as showing evidence for modern behaviour in a piece-meal (non-linear) fashion.

This latter scenario may be consistent with the fossil and genetic record, as now understood. The fossil and genetic evidence point to a very complex modern human origin: with both extensive migration, and hybridization between expanding populations of AMHs and archaics. Modern and ancient DNA points to a largely recent African origin, as common among all modern humans, but introgression was occurring frequently, with numerous archaic lineages represented within contemporary modern human populations. Recent finds, such as *Homo naledi* and *Homo floresiensis*, greatly increase the range of morphological variation of hominins in the Middle and Late

Pleistocene (Brown *et al.* 2004, Berger, Hawks *et al.* 2015). Furthermore, the association of small brained hominins with potential burial and sophisticated stone tool technologies, implies that large morphological variation, including small brain size, does not necessarily mean advanced cognitive abilities were limited to certain populations. A highly variable fossil record in the Middle and Late Pleistocene provide us with greater numbers of lineages or taxa from which to draw potential introgression both outside and within Africa. Thus, both the fossil and cultural record could point towards a more complex evolution of our species, cognitively and morphologically (Ackermann, Mackay *et al.* 2016).

BEYOND THE LATE PLEISTOCENE

While hybridization is easier to conceptualize within the context of the late Middle to Late Pleistocene, where there are numerous known hominin taxa which are morphologically diverse and geographically expansive, it is quite possible that hybridization occurred among hominins even deeper in time. Among Old World Monkeys, hybridization has been seen in very recently-diverged taxa, as well as intergeneric (see previous chapter). This implies hybridization may occur despite almost 2 Ma of divergence among lineages, although it is important to note that speciation between taxa is complex.

Ackermann (2010) points out that evidence for hybridization may occur earlier in the hominin fossil record, as indicated by the presence of signatures of developmental disruption. SK 83, a *Parathropus robustus* from South Africa, has a supernumerary maxillary lateral incisor (Ripamonti, Petit *et al.* 1999, Ackermann 2010). However, this trait, although uncommon, is not observed in the current hybrid morphological literature (and is among most common of the supernumerary dentition in humans). A.L. 198-1, an *Australopithecus afarensis* from Ethiopia, displays the roots of a supernumerary mandibular molar (White, Johanson 1982, Ackermann 2010), arguably a better signature (the trait is observed in baboon F1 males, and is incredibly rare in modern human populations; Ackermann, Schroeder *et al.* 2014, Ackermann, Rogers *et al.* 2006). While neither of these specimens can be proven to be hybrids, it is important to note the diversity of hominin taxa living within southern and eastern Africa at these time periods makes hybridization among distinct lineages possible. It is also useful to realise that at least a few of these taxa may have been living around the same time: for instance, the discovery of a *Homo erectus* and *Paranthropus boisei*

skeletal elements in a similar layer at Koobi fora (Leakey, Walker 1976), although a better example may be the hominin diversity in East Africa between 4-3 Ma (Wood, K Boyle 2016).

Evidence for gene flow among even earlier hominins and chimpanzees has been proposed on the basis of the discordance within the genomes of humans and other great apes (Patterson, Richter *et al.* 2006, Scally, Dutheil *et al.* 2012). Molecular studies have calculated a human-chimp divergence of between 4-6 Ma and a human-chimpanzee-gorilla divergence at around 6-10 Ma (Castresana 2001, Wall 2003). However, the chimp-human divergence-estimate does not match the paleoanthropological record, where *Sahelanthropus tchadensis* (from Chad) is interpreted as being hominin (through the dentition and some evidence for bipedalism) and dated to around 6.4 Ma (Brunet, Guy *et al.* 2002, Brunet, Guy *et al.* 2005), and *Orrorin tugenensis* (from Kenya) has been dated to around 6 Ma (Senut, Pickford *et al.* 2001, Pickford, Senut 2001). Patterson and colleagues highlighted discordance/variance in the human-chimp genetic divergence estimates throughout the genome: a divergence difference of around 4 million years, with the X chromosome being particularly recent (Patterson, Richter *et al.* 2006). This evidence, they say, points to a more recent hybridization event since the original divergence. However, the models used and interpretation of this research may also be explained by selection, adequately large and variable founder populations, and variable mutation rates over the genome and over time: all consistent with allopatric speciation (Barton 2006, Wakeley 2008, Presgraves, Soojin 2009).

Accumulations of mtDNA into nuclear genomes (NUMTs) appear to have increased in insertion rate roughly 2.8 Ma in humans: a date consistent with the emergence of our genus (Gunbin, Peshkin *et al.* 2016). It is possible that hybridization among genetically divergent individuals may have facilitated the increase in NUMT insertion (Gunbin, Peshkin *et al.* 2016).

Further molecular research showed that gorillas are closer to chimps and humans in roughly 30% of the genome than either were to each other (Scally, Dutheil *et al.* 2012). Close contact between human and gorilla ancestors (albeit, not necessarily hybridization) may be evidenced in lice divergence. Phylogenetic research on lice has shown that human pubic lice diverged from gorilla lice around 3-4 Ma, yet the estimated divergence date between human (head and body) lice and chimpanzee lice compares with that of the calculated human-chimp divergence (6 Ma; Reed, Light *et al.* 2007). It therefore seems likely that human ancestors acquired pubic lice from gorillas. While human public lice are generally transferred via sexual contact, there may be other explanations for host transmission, such as overlapping nesting areas (Reed, Light *et al.* 2007).

SUMMARY

Within this section, past debates and hypotheses concerning human origins have been broadly summarised. Because of advances in ancient DNA sequencing, many researchers do not subscribe fully to either complete RAO or to regional continuity. Our current understanding is more complex, with evidence supporting a largely Out of Africa scenario, but with hybridization occurring among many interacting hominins. While modern human genomes derived from Africa quickly swamped those of hominins in Eurasia in the Late Pleistocene, interaction was rampant, and these hominins have left large proportions of their DNA (albeit only a small proportion per individual) in peoples all over the world today. Similarly, while genetic evidence is more scarce, or non-existent, among hominins deeper into the past, it is likely that a highly diverse taxa, of recent divergence, were hybridizing in at least some instances.

Purpose: *To expand and explain the rationale for choosing certain Mus strains to understand hybridization in mammals. To describe the methodologies employed in the three subsequent results chapters.*

CHAPTER 5

MATERIALS AND METHODS

MATERIALS

MOUSE STRAINS USED

Mice, particularly of the genus *Mus*, are particularly effective taxa for understanding hybridization. There are multiple species and subspecies that hybridize, with varying time depths for divergence. Geographically, there are taxa that are completely allopatric, and those that are sympatric. There are those that hybridize in the wild, and those that (due to either geographic distance, or post- and pre-zygotic barriers) do not. Within the genus *Mus* (and the subgenus of the same name), there are several species that diverged and radiated over Eurasia roughly 1.6-1 Ma: *Mus sprecilegus*, *M. musculus*, *M. spretus* and *M. macedonicus* (the latter two are more closely related sibling taxa; She, Bonhomme *et al.* 1990, Boursot, Auffray *et al.* 1993, Suzuki, Shimada *et al.* 2004). Among the house

mouse, *Mus musculus*, there are numerous subspecies that diverged 600-500 Ka, and occur mostly allopatrically, with hybrid zones of varying success (She, Bonhomme *et al.* 1990, Boursot, Auffray *et al.* 1993, Duvaux, Belkhir *et al.* 2011, Phifer-Rixey, Nachman 2015, Geraldes, Basset *et al.* 2008, Geraldes, Basset *et al.* 2011). Although the taxonomic designation of some of these *M. musculus* subspecies changes regularly with increasing genetic information, several are fairly constant (but see Carleton, Musser 2005), and will be the main subjects of this study.

Four parental mouse strains were bred for use in this research. They represent four distinct mouse taxa (different species or subspecies) from the genus *Mus*. All mice were housed and bred at the University of Calgary in accordance with approved animal care protocols from both the University of Calgary and the University of Cape Town (AC1-0210 and 2012V56RA, respectively).

The three subspecies (sometimes referred to as species) of house mice (the species *Mus musculus*) used in these analyses are: *M. m. musculus* (represented by the Jax strain, CZECHI/EiJ, hereafter abbreviated to CZE), *M. m. domesticus* (represented by the Jax strain, WSB/EiJ, hereafter abbreviated to WSB) and *M. m. castaneus* (represented by the Jax strain, CAST/EiJ, hereafter abbreviated to CAS). These subspecies diverged from each other approximately 600 Ka, in the Fertile Crescent, according to phylogenetic analyses (She, Bonhomme *et al.* 1990, Boursot, Auffray *et al.* 1993). Each of these strains developed commensalism separately, and, through commensalism, spread to northern Eurasia (*M. m. musculus*/CZE), western Europe (*M. m. domesticus*/WSB) and southeast Asia (*M. m castaneus*/CAS) after the dawn of agriculture in Eurasia (Boursot, Auffray *et al.* 1993, Bonhomme, Searle 2012). Of these, *M. m. domesticus* (WSB) has travelled with humans outside of the Old World into the Americas and Australia, following European colonisation of those continents. The subspecies used here are the best described of the *M. musculus*, with the most extensive geographic expanse (see Figure 5.1).

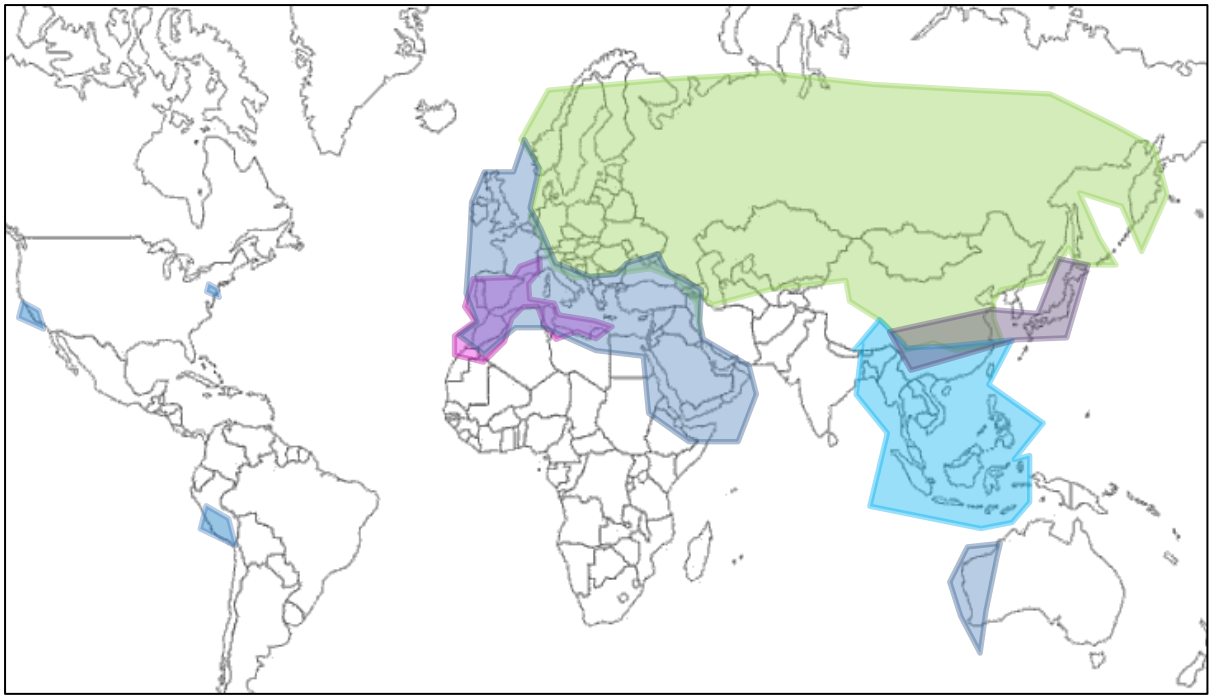

FIGURE 5.1. GEOGRAPHIC DISTRIBUTIONS OF THE MICE USED IN THIS THESIS (IMAGE ADAPTED FROM KOZAK 2014); *M. M. DOMESTICUS* (WSB) IN DARK BLUE, *M. M. MUSCULUS* (CZE) IN GREEN, *M. M. CASTANEUS* (CAS) IN LIGHT BLUE, AND *M. SPRETUS* (SPR) IN PINK. ALSO DEPICTING *M. M. MOLOSSINUS* IN PURPLE.

Secondary contact zones have since occurred between these species, which have been briefly discussed in previous chapters. Between *M. m. musculus* (CZE) and *M. m. domesticus* (WSB), a narrow hybrid zone exists through Belgium and France (Auffray, Alibert *et al.* 1996, Dod, Jermiin *et al.* 1993, Alibert, Renaud *et al.* 1994, Bonhomme, Searle 2012, Boursot, Auffray *et al.* 1993, Moulia, Aussel *et al.* 1991, Mikula, Auffray *et al.* 2010, Baird, Macholan 2012, Macholan 1996, Teeter, Payseur *et al.* 2008, Teeter, Thibodeau *et al.* 2010). Hybrids in this zone exhibit low fertility and viability, yet hybridization still occurs, and gene flow across the hybrid zone still influences genotypic variation in the parent populations on either side of it (Teeter, Payseur *et al.* 2008, Payseur, Krenz *et al.* 2004, White, Stubbings *et al.* 2012, Teeter, Thibodeau *et al.* 2010). Furthermore, despite clear lack of hybrid fitness (greater inviability, infertility and higher pest load), the hybrid zone appears to be maintained by selection (Dod, Jermiin *et al.* 1993, Ting, Tsaur *et al.* 1998, Teeter, Payseur *et al.* 2008). In contrast, a large hybrid zone occurs between *M. m. musculus* (CZE) and *M. m. castaneus* (CAS) in China and Japan. In this hybrid zone, the hybrid mice are highly successful and appear better-adapted to commensal living than the species from which they are derived (Yonekawa, Moriwaki *et al.* 1988, Yonekawa, Sato *et al.* 2012, Shurtliff 2013). This was the first recorded

mammalian hybridization-derived taxon, named *M. m. molossinus*. There is no natural hybrid zone between *M. m. domesticus* (WSB) and *M. m. castaneus* (CAS); several other described *M. musculus* subspecies occur between the two subspecies in southern Asia. We thus have three differing hybrid scenarios among these subspecies: one where the hybrid is less fit, yet selected for in nature, one where the hybrid is fitter than the parents and forms a natural hybrid taxon, and one where hybridization does not occur in nature.

The strains used are all inbred and wild-derived. While there are issues with using inbred strains, they best represent isolated populations far from hybrid zones. The specific strains used for this study (and the Mouse Hybrid Project as a whole) were chosen due to the proportion of the genome representing a single sub-specific origin (Yang, Wang *et al.* 2011). Wild-derived mice are also extensively used in evolution and systematics research, and are comparable with wild-caught mice in having large numbers of naturally-derived polymorphisms, and similar behaviours and levels of infection-resistance (Guénet, Bonhomme 2003). CZE (representing *M. m. musculus*) was derived from wild-caught mice from Slovakia. WSB (representing *M. m. domesticus*) was derived from wild-caught mice in Maryland, USA. CAS (representing *M. m. castaneus*) was derived from wild-caught mice in Thailand (for reference, see the JAX database). All three strains used here were trapped far outside of any potential intraspecific hybrid zone, and are therefore good representations of their subspecies, with lower potential influence from selected introgressed genes from other subspecies which may influence the phenotype.

In addition to *M. musculus* subspecies, one other species was bred: *M. spretus* (represented by the Jax strain SPRET/EiJ, hereafter SPRET), and interspecific hybridization was attempted between this taxon and *M. musculus*. SPRET was derived from wild-caught mice in Spain (see JAX database). SPRET are field mice, and are non-commensal with humans. They therefore typically inhabit very different ecological niches from the House Mice described earlier. *M. spretus* displays low levels of agonistic behaviour in both males and females (Frynta, Slabova *et al.* 2005).

Three different intraspecific F1 hybrids were bred: CASxCZE (hybrid between *M. m. castaneus*, CAS, and *M. m. musculus*, CZE), CASxWSB (between *M. m. castaneus*, CAS, and *M. m. domesticus*, WSB), and CZExWSB (between *M. m. musculus*, CZE, and *M. m. domesticus*, WSB). While F1 hybrids were typically viable, male hybrid infertility between *M. m. musculus* (CZE) and *M. m. domesticus* (WSB), meant that only the female CZExWSB F1 hybrids were able to produce offspring. Therefore, there are no CZExWSB F2s, and all CZExWSB backcrosses were with F1 hybrid dams. It was possible to breed F2s from both the CASxCZE and CASxWSB F1 strains. Backcrosses between F1 hybrids into parents were also bred for all three crosses.

Mus interspecific hybrids are incredibly rare. *M. spretus* (SPRET) is smaller and displays higher levels of stress than *M. musculus* mice, yet they may interbreed (Dejager, Libert *et al.* 2009). Thus it was possible to cross *M. spretus* with *M. musculus* in this study, and the only interspecific hybrid that will be studied is between the strains SPRET and WSB. Natural hybrids between these taxa are rarely reported and, while it is possible in the wild, certain factors appear to support inviability: hybrid male infertility (consistent with Haldane's Law), and that F1 hybrids themselves only result from male *M. spretus* and female *M. musculus* crosses (Dejager, Libert *et al.* 2009). Therefore crossing them here is interesting for two reasons: 1) despite the fact that these species exist sympatrically (geographically, albeit generally occupying different ecological niches), hybridization and gene flow rarely occurs; and 2) evidence for gene flow is only associated with genes with very high adaptive success, thus governed strongly by selection (see Chapter 2).

The list of strains bred and used in this thesis is in Table 5.1.

TABLE 5.1. STRAINS AND CROSSES OF MUS USED IN THIS THESIS.

Strain	Type	Cross	Scientific name and notes	Total n
CAS	Parent	CAS	*M. m. castaneus*	50
CZE	Parent	CZE	*M. m. musculus*	50
WSB	Parent	WSB	*M. m. domesticus*	50
SPRET	Parent	SPRET	*M. spretus*	50
CZExWSB	F1 hybrid	CAS and CZE	Haldane's Rule applies; natural hybrid zone in Europe	50
CASxWSB	F1 hybrid	CAS and WSB	No natural hybrid zone	50
CASxCZE	F1 hybrid	CZE and WSB	Natural hybrid zone in east Asia with hybrid species, *M. m. molossinus*.	50
SPRxWSB	F1 hybrid	SPRETUS and WSB	Interspecific cross; Haldane's Rule applies	36
CASxCZE_F2	F2 hybrid	CASxCZE		50
CASxWSB_F2	F2 hybrid	CASxWSB		50
(CASxCZE)xCZE	B1 hybrid	CASxCZE and CZE		50
(CASxWSB)xCAS	B1 hybrid	CASxWSB and CAS		50
(CZExWSB)xWSB	B1 hybrid	CZExWSB and WSB	WSBxCZE dams only (due to Haldane's Rule)	50

The combined parent strains and hybrids will be referred to throughout this thesis as a "group", as opposed to "strain", which will be used to refer to individual genotypes. i.e. SPRETUS is a strain which is combined with WSB and the SPRxWSB F1 hybrid to form the SPR/WSB group.

SCANNING AND LANDMARKING

Mice older than 90 days were sacrificed and MicroCT scanned at the University of Calgary using a Scanco vivaCT40 microCT scanner at 0.035mm voxel size at 55kV and 72-145µA. For the sake of consistency across broader mouse studies (outside of the scope of this work), and to reduce inter-user error across these studies, cranial landmarks were collected by the laboratory technician, Wei Lui, at the University of Calgary. All cranial landmarks were collected using Analyze 3D (version 5.0). These landmarks were chosen to be consistent with other studies in the U Calgary lab, and with previous publications (see Percival, Liberton *et al.* 2016). Mandibular landmarks were collected by Kerryn Warren (author), using the program Meshlab version 1.3.2. These mandibular landmarks are derived from Willmore and colleagues (2009), Olson colleagues (2004), and landmarks used by the Richtsmeier Laboratory (http://getahead.psu.edu/viewer.html?id=Adult_Mouse_Mandible). Cranial and mandibular landmarks are listed in Table 5.2 (adapted from Warren *et al.* in review) and Figure 5.2.

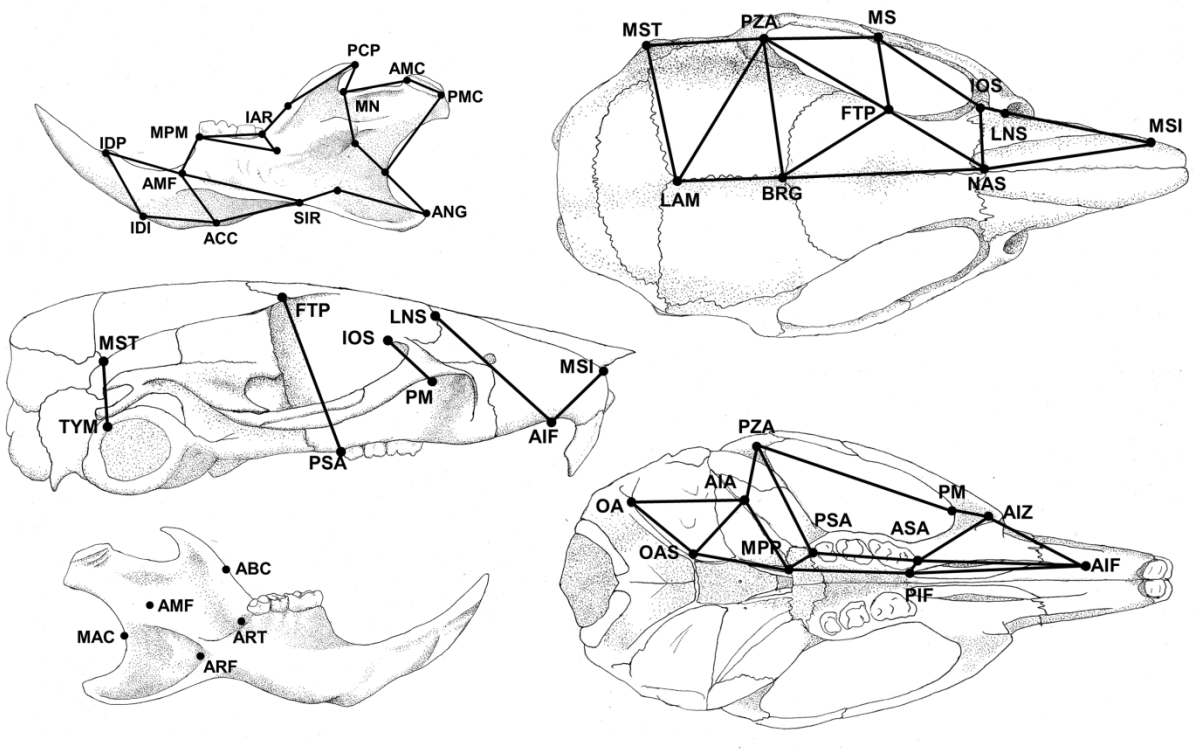

FIGURE 5.2. LANDMARKS AND INTER-LANDMARK DISTANCES USED FOR ANALYSIS IN THIS THESIS.

TABLE 5.2. CRANIAL AND MANDIBULAR LANDMARKS USED IN THIS THESIS (ADAPTED FROM WARREN *ET AL*. IN REVIEW).

Landmark anagram	Landmark name
	Cranial landmarks
MSI	Midline superior incisor
AIF	Ant. margin of incisive foramen
AIZ	Ant. inferior zygomatic
PM	Point of greatest curvature on the posterior margin of the malar process
ASA	Ant. superior alveoli
PIF	Post. incisive foramen
PPS	Point along palatine-maxillary suture
PSA	Post. superior alveoli
SOS	Spheno-occipital synchondrosis
AFO	Ant. foramen ovale
AIA	Ant. inferior auditory bulla
PZA	Point of greatest curvature along posterior edge of zygomatic process of temporal bone
OAS	Occipital-auditory-sphenoid junction
OA	Occipital-auditory junction
ATS	Auditory-temporal-sphenoid junction
MPP	Med. palatal-pterygoid junction
MMP	Med. maxilla-premaxilla junction
LNS	Anteriormost point along lateral zygomatic-frontal suture
NAS	Nasion
LFS	Lateral point along frontal suture
IOS	Intersection of frontal suture with orbital rim
MS	Superior margin of suture of temporal and zygomatic processes of zygomatic arch
FTP	Frontal-temporal-parietal junction
BRG	Bregma
LAM	Lambda
MST	Point along occipomastoid suture
TYM	Superoposterior extremity of tympanic ring
PTZ	Post. temporal-zygomatic junction
ATZ	Ant. temporal-zygomatic junction
PZF	Post. zygomatic-frontal junction
	Mandibular landmarks
MPM	Anterior edge of alveolar process where first molar hits alveolus at the midline
IAR	Intersection of molar alveolar rim and base of coronoid process
ACC	Anterior edge of the coalescence of curve of masseteric ridge with post-symphyseal rugged area
IDP	Superior-most point on incisor alveolar rim at midline (at bone-tooth junctions)
PCP	Apex of coronoid process
ANG	Tip of mandibular angle
AMC	Anterior midline point on condyle
PMC	Posterior midline point on condyle
IDI	Inferior-most point on incisor alveolar rim at midline (at bone-tooth junction)
SIR	Superior-most point on inferior border of mandibular ramus
AM	Anterior edge of the mental foramen
ARF	Anterior edge of the ramal fossa foramen. If two foramina, use lower foramen
ART	Apex of retromolar trigone
PBC	Posterior base of coronoid process at midline
ABC	Anterior base of coronoid process at midline
AMF	Anterior edge of mandibular foramen
MAC	Most concave point on subcondylar incisive

METHODOLOGY

ANALYSIS FOR CHAPTER 6

These analyses were designed in order to be consistent with previous studies conducted on hybrid baboons, gorillas and tamarins (Ackermann, Schroeder *et al.* 2014, Ackermann, Rogers *et al.* 2006, Ackermann, Bishop 2010, Cheverud, Jacobs *et al.* 1993). Many of the analyses used in this section are described in Warren *et al.* (in review), which is included as Chapter 6, although there are several preliminary tests which are described here in greater detail.

Because different sections of the results were completed at different times throughout the breeding process, sample sizes vary among the analyses; this variance will be recorded throughout the chapter. Similarly, some strains were already bred to a full sample size complement before others. Since this was the first analysis performed for the Mouse Hybrid Project, within this section intraspecific parent strains are compared with the F1 hybrids. Multigenerational crosses and the interspecific cross will be expanded upon in the next section (describing methodologies used in Chapter 7).

FORM

To investigate cranial and mandibular form, 38 interlandmark distances were calculated from the cranial landmark coordinates and 21 from the mandibular landmarks (left side; Figure 5.2). These interlandmark distances were chosen to provide complete coverage of the mandible and cranium while minimizing redundancy. To investigate cranial and mandibular form, all interlandmark distances were compared among all groups in the sample (i.e., all three parent strains and all three hybrids) using a MANOVA in PAST. Form differences were also assessed by comparing all interlandmark distances among the three parent strains and among the three F1 hybrids, using T-tests. In addition, comparisons were made for each parent-parent-hybrid set.

Shapiro-Wilks tests and Levene's tests of the interlandmark distances suggested significant deviations from normality and homogeneity of variance, respectively. These deviations were not consistent among interlandmark distances for all groups—i.e. some distances deviated from normality in some, but not all groups and heterogeneity of variance was present in only some distances. Reasons for these deviations might include post-maturation growth (which affect groups

with more younger or older individuals), different levels of sexual dimorphism, and/or differences in maternal effect (where the phenotype is further influenced by the mother during pregnancy and lactation) among groups. Tests were done to better calculate which of these potential effects may influence normality, but, once again, there was inconsistency in which distances were affected by which strain. Some landmarks were therefore normally distributed in one parent, with kurtosis in another, and/or bimodal in the hybrid of the two, making it impossible to correct for these deviations consistently among all three groups. Due to these deviations, analyses of the interlandmark data were initially conducted using non-parametric tests, yet in direct comparisons, student's T-tests proved most robust (Skovlund, Fenstad 2001).

SIZE

Geometric means of the cranium and mandible were computed for each individual, and these geometric means were compared between a combined sample of all parent specimens and a combined sample of all hybrid specimens, using T-tests. In addition, the geometric means were compared in each parent-parent-hybrid group. An analysis of heterosis of overall cranial size was also performed using the geometric means of the interlandmark distances. Within this thesis we will use the term "heterosis" to describe measures in hybrids which are significantly larger than the expected midparental value (MPV). Specifically, the MPV was calculated for each pair of parents by computing the average of their geometric means (as per Bruell 1964). Because these data indicated deviations from normality and homogeneity of variance (see above), MPVs for each pair of parents were then compared to the 95% confidence intervals of bootstrapped distributions (9,999 permutations) of means of the respective hybrids.

Tests of heterosis were also performed for each of the interlandmark distances. These tests were conducted in the same way as those for overall cranial size—i.e. MPVs for each pair of parents for each interlandmark distance were computed and compared to 95% confidence intervals of a boot-strapped distributions of means from their respective hybrid.

SHAPE

Shape of the skull and mandible in the parents and F1s was analysed using Principal Components Analysis (PCA) on the Procrustes-aligned landmarks, in the programme MorphoJ. The PCAs were primarily used for visual interpretation of shape differences most affecting separation of individuals.

ANALYSIS FOR CHAPTER 7

Within chapter 7, we shall explore cranial and mandibular size and shape variation in the mice and their hybrids. Unlike in Chapter 6 we will focus on geometric morphometric techniques, using the program, MorphoJ, and various packages (including Geomorph) in the statistical program, R, to analyse landmark data directly. Within this chapter we have also included multigenerational recombinants in our analyses, looking at one backcrossed lineage (B1) in all intraspecific crosses (hybrid backcrossed with one parent), and second generation hybrids (F2) from CAS/CZE and CAS/WSB (F1 hybrids crossed together). Sample sizes used in this section are presented in Table 5.3. This differs from those used in Chapter 6 due to the collecting of data at different stages of completion of sections of this thesis (for publication), differential levels of fertility of breeding strains and availability of scans.

TABLE 5.3. SAMPLE SIZES USED IN CHAPTER 7 ANALYSES.

	Cranium	Mandible
CAS	50	30
CZE	50	30
WSB	49	29
SPRET	50	0
CASxCZE	50	30
CASxWSB	47	30
CZExWSB	50	29
SPRxWSB	36	0
CASxCZE_F2	30	30
CASxWSB_F2	30	29
(CASxCZE)xCZE	29	6
(CASxWSB)xCAS	30	29
(CZExWSB)xWSB	30	20

Within these analyses, a fuller set of cranial landmarks was used, in order to better interpret modules in later analyses. In initial analyses, the error associated with one of the landmarks (Lateral palatal-pterygoid junction), was too great, and was therefore eliminated from the dataset prior to analyses. Thus, in total, 30 landmarks were used, 27 of which were bilateral. The cranial and mandibular landmarks were collected as described earlier.

The cranial and mandibular data were imported into MorphoJ (v1.06a; Klingenberg 2011) and subjected to Procrustes fit. The shape data were treated differently for the cranium and mandible because they are datasets with two different kinds of symmetry. The cranial data exhibit object symmetry. Within the programme, MorphoJ, object symmetry is taken into consideration during the Procrustes fit. All analyses described (except Procrustes ANOVA) will be performed on the symmetrical component of this shape data. The mandibular data however is collected on a structure (the mandible) where the two sides of the structure are not always firmly connected. These are then treated as having matching symmetry, where the data for both the left and right sides of the mandible are reported separately. In these analyses, the data for mandibular left and right halves are averaged per individual.

Measurement Error

In order to better understand intra-observer error inherent in the datasets, two separate analyses were performed for the cranial and mandibular landmarks. Since data for the cranial landmarks within the hybrid dataset were only collected once, a Procrustes ANOVA was performed on cranial landmark data collected in a previous study by the same person (Wei Lui). Within this subset 101 mice from several different strains, and their hybrids, were landmarked twice. For the mandibular data, a subset of 55 individuals (5 per strain) was landmarked twice (i.e. one re-collection).

Table 5.4 shows the results of the Procrustes ANOVA for the cranial analysis. This is a separate group of mice from that used in the study, and many more strains of mice were assessed in this dataset. Therefore, strain-association is not used as a covariate in this analysis. The Procrustes ANOVA shows that the replication error for shape is small compared with individual and side covariates. This means that analyses on individuals and directional asymmetry is possible. However, the relatively low mean squares and F-value for the fluctuating asymmetry of shape (Ind*Side) indicates that it is not advisable to use these data to study fluctuating asymmetry. For centroid size, replication error is far smaller than differences among individuals.

TABLE 5.4. RESULTS OF THE CRANIAL PROCRUSTES ANOVA.

Centroid size:					
Effect	**SS**	**MS**	**df**	**F**	**P (param.)**
Individual	762.864	7.70570	99	455.77	<.0001
Replication	1.691	0.01691	100		
Shape, Procrustes ANOVA					
Effect	**SS**	**MS**	**df**	**F**	**P (param.)**
Individual	0.1861	0.000023	8019	6.85	<.0001
Side	0.0132	0.000178	74	52.63	<.0001
Ind * Side	0.0248	0.000003	7326	0.98	0.8847
Replication	0.0538	0.000003	15500		

TABLE 5.5. RESULTS OF THE MANIDIBULAR PROCRUSTES ANOVA.

Centroid size:					
Effect	**SS**	**MS**	**df**	**F**	**P (param.)**
Group	64.8099	6.48099	10	13.1	<.0001
Individual	21.7707	0.49479	44	46.02	<.0001
Side	0.06775	0.06775	1	6.3	0.0151
Ind * Side	0.58063	0.01075	54	2.02	0.0009
Replication	0.58506	0.00532	110		
Shape, Procrustes ANOVA					
Effect	**SS**	**MS**	**df**	**F**	**P (param.)**
Group	0.24794	0.00056	440	7.7	<.0001
Individual	0.14168	0.00007	1936	2.42	<.0001
Side	0.04148	0.00094	44	31.22	<.0001
Ind * Side	0.07174	0.00003	2376	0.96	0.8443
Replication	0.15151	0.00003	4840		

SIZE ANALYSES

Several techniques were used to understand cranial and mandibular size variation between strains within groups. Centroid sizes for the crania and mandibles were analysed separately. An ANOVA analysis and Tukey Tests were performed on the datasets to understand size differences between group means. A Levene's Test was used to quantify potential differences in variance between the centroid sizes of strains.

In order to assess whether cranial or mandibular size alone is an appropriate metric for hybridization within or between groups, these centroid sizes were used in a simple classification analysis. Within the analysis, absolute differences between the centroid size of the individual from averages of the parents and F1 hybrids were calculated. The individual was then "assigned" to the group to which the difference in centroid size is smallest. This measure is simple, and flawed in several ways. Firstly, it assumes differences among strains, which may actually overlap greatly. Secondly, it does not take into consideration variation in group size. This means that individuals of strains with large size variation are more likely to be placed in another strain. However, in order to better understand the extent to which hybrid heterosis is retained in subsequent generations, such simple metrics may prove useful. In order to better understand the power of this classification technique, individuals in the parent and F1 strains were also classified.

Similarly, heterotic size may be seen as a powerful indicator of hybridization within a population. However, it is unlikely that a single population will have only F1 hybrid phenotypes (including heterosis). It is therefore important to understand the extent to which hybrid morphologies may influence the mean and variance of cranial and mandibular size of a sample. A simple mixed model analysis is performed, where different proportions of hybrids and parents are sampled (999 times per proportion). Resampling takes place at 0% hybrids (whereby samples are taken from parents only, and both parents are sampled together), and 10% hybrid increments until only hybrids are sampled in each group (samples are 100% hybrids). Parents are pooled in each group to better determine the difference in size variation between sympatrically-occurring groups that are hybridizing, and those that are not hybridizing.

The classification analysis and the mixed model analysis should together give a better understanding of the extent to which cranial or mandibular size are useful indicators of hybridization in a population.

Shape Analyses

In order to understand and explore the effects of hybridization on cranial and mandibular shape, each analysis was performed separately for each parent-parent-hybrid group; three intraspecific parents plus F1 hybrid (CAS/CZE, CAS/WSB and CZE/WSB) groups, and one interspecific parents plus F1 hybrid (SPR/WSB) group, were analysed. The three intraspecifc hybrid groups will be presented together for each analysis, so that trends in shape among hybrids of similarly divergent lineages may be better visualized.

To explore shape variation, a PCA for each group was performed, based on the covariance matrix of the symmetric shape coordinates. This is done in order to see what shape change is associated with the main features of variation among individuals, and whether these features also separate out hybrid and parent groups.

Since allometry is likely to have an effect on these data, regression analyses were also performed on each of the groups (shape on size). Another PCA was then performed on the pooled-within group regression residuals.

Differences between groups

Procrustes and Mahalanobis distances between groups were also computed, as well as a canonical variates analysis in MorphoJ. Shape change along canonical variates was also observed. Similarly, the Procrustes variance of observations for each strain was computed in R, using the General Procrustes Analysis (GPA) coordinates generated in MorphoJ.

A similar classification analysis to that conducted during the size analyses was performed on shape data, using Procrustes distances. Procrustes distances between individuals (regardless of strain) and the Procrustes mean of the parent and F1 hybrid strains were calculated. The individuals were then classified into the strain (parent or F1 hybrid) with which the smaller Procrustes distance was calculated. This analysis has similar faults to those seen above: it is assumed that the Procrustes means of strains are different enough to appropriately classify individuals, and that variation within a strain will not create too much overlap among individuals in strains. However, this analysis is useful in understanding the extent to which hybrid or parent morphologies are present in multigenerational recombinants. In order to better understand the power of this classification technique, individuals in the parent and F1 strains will also be classified.

Integration and Modularity

Two analyses were performed to better understand integration and modularity in the skull and mandible of parent and hybrid strains. It is useful to understand whether some of the differences seen between parents and hybrids may be due to a breakdown in integration of these structures. Because correlation analyses lose power when there are more variables than individuals in each sample, modularity was hypothesized first, and then these modules were used to look at covariation among each module between strains.

TABLE 5.6. PARTITIONS USED IN MODULARITY ANALYSIS.

Landmark	Assigned region	Landmark	Assigned region
Cranium		**Mandible**	
MSI	Facio-palatal region	MPM	Anterior
AIF	Facio-palatal region	IAR	Anterior
AIZ	Facio-palatal region	ACC	Anterior
PM	Facio-palatal region	IDP	Anterior
ASA	Facio-palatal region	PCP	Posterior
PIF	Facio-palatal region	ANG	Posterior
PPS	Facio-palatal region	AMC	Posterior
PSA	Facio-palatal region	PMC	Posterior
SOS	Basicranium	IDI	Anterior
AFO	Basicranium	SIR	Anterior
AIA	Basicranium	AM	Anterior
PZA	Neurocranium	ARF	Posterior
OAS	Basicranium	ART	Anterior
OA	Basicranium	PBC	Posterior
ATS	Basicranium	ABC	Posterior
MPP	Facio-palatal region	AMF	Posterior
MMP	Facio-palatal region	MAC	Posterior
LNS	Facio-palatal region		
NAS	Facio-palatal region		
LFS	Facio-palatal region		
IOS	Facio-palatal region		
MS	Facio-palatal region		
FTP	Neurocranium		
BRG	Neurocranium		
LAM	Neurocranium		
MST	Neurocranium		
TYM	Neurocranium		
PTZ	Neurocranium		
ATZ	Neurocranium		
PZF	Facio-palatal region		

The mandible was separated into two modules and the cranium was separated into two and three modules (Table 5.6; Figure 5.3). For the mandible, these modules represent the anterior part of the mandible (which includes the alveolar morphology and the body) and the posterior part of the mandible (representing the condyles, coronoid process: regions on the mandible which could be most affected by musculature involved in chewing). Modules hypothesized in cranium is separated

into the face, neurocranium and basicranium (three part modularity test), and the face and cranium (combined neurocranium and basicranium; two modules), to test whether these modules exhibit weaker covariance than for other random partitions (Klingenberg 2008). The RV coefficient (Escoufier 1973) for the partition will be compared with the distribution of other coefficients for 10 000 ranWSB partitions.

Correlation tests between covariance matrices are performed between the strains for each of the above modules which show some degree of modularity in the tests. This allows for better interpretation of where disintegration may occur within hybrids and multigenerational recombinants. Furthermore, it reduces the error inherent in having more variables than specimens for each comparison. The methodology used has been adapted for geometric morphometrics (Klingenberg 2008, Klingenberg, Barluenga *et al.* 2002).

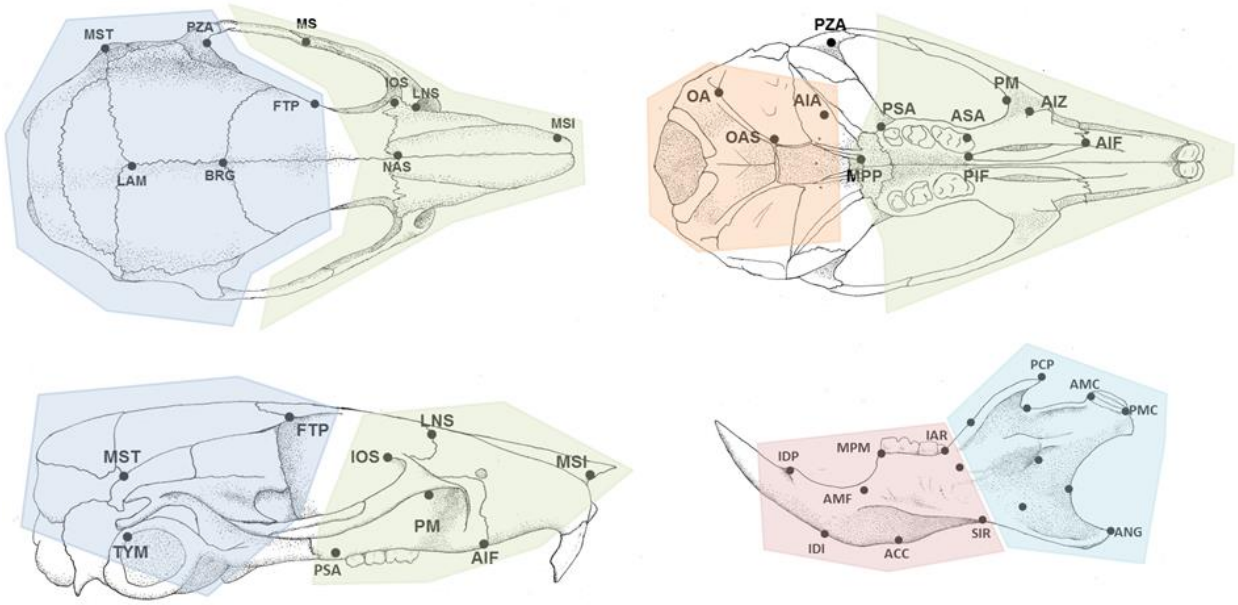

FIGURE 5.3. MODULES USED IN THIS THESIS. FOR THE CRANIUM: NEUROCRANIUM (DARK BLUE), FACIO-PALATAL (GREEN), AND BASICRANIUM (ORANGE). FOR THE MANDIBLE: ANTERIOR (PINK) AND POSTERIOR (LIGHT BLUE).

ANALYSES FOR CHAPTER 8 (NON-METRIC TRAITS)

PROPOSED TRAITS

One noted feature in the literature on hybrid morphologies is that of unusual non-metric trait variation. In the final results chapter, non-metric trait variation in the cranio-mandibular morphology of the parent mice and their F1 hybrids was quantified. Previous studies have found a preponderance of atypical dental and sutural morphologies (extra sutures and ossicles) in hybrid groups (Brink 2005, Ackermann, Schroeder *et al.* 2014, Ackermann, Brink *et al.* 2010, Ackermann, Rogers *et al.* 2006, Ackermann, Bishop 2010, Ackermann 2009). Therefore an emphasis is placed on examining variation in these features. Furthermore, foramina and hyperstotic features were also observed. All features are recorded and described in Table 5.7.

Below is a full list of examined traits. They are grouped either by the structure on which they are to be found, or by the kind of feature observed. The list also includes features which were examined, but ultimately excluded due to poor visibility on many of the scans.

TABLE 5.7. FEATURES OBSERVED FOR NON-METRIC TRAIT VARIATION (NOT BILATERAL = NOT B).

Features
Teeth
- Supernumerary (>1URI, >1ULI, >1LRI, >1LLI, >3URM, >3ULM, >3LRM, >3LLM)
- Description if supernumerary: conical, tuberculate, supplemental, odontomas; and bilateral (Ackermann, Rogers *et al.* 2006)
- Rotated teeth (Ackermann, Rogers *et al.* 2006)
Suture Fusion
- Nasal fusion (not B; Berry, Searl 1963, Richtsmeier, McGrath 1986)
- Squamosal-frontal fusion (Berry, Searl 1963, Richtsmeier, McGrath 1986)
- Squamosal-parietal fusion (Richtsmeier, McGrath 1986, Searle 1954)
- Post tympanic hook (Richtsmeier, McGrath 1986)
- Basisphenoid-basioccipital (not B; Berry, Searl 1963, Richtsmeier, McGrath 1986)
- Basisphenoid-presphenoid (not B; Richtsmeier, McGrath 1986, Deol, Truslove 1957)
- Preoptic root (Truslove 1954, Richtsmeier, McGrath 1986)
- Dorsal frontal fusion (not B; Richtsmeier, McGrath 1986)
- Occipital-pteriotic fusion (Richtsmeier, McGrath 1986)
Extra sutures
- Nasal
- Premaxilla
- Maxilla (Ackermann, Rogers *et al.* 2006)
- Frontal
- Zygomatic (Ackermann, Rogers *et al.* 2006)
- Squamosal
- Parietal

- Interparietal
- Occipital
- Palatine
- Basisphenoid
Ossicles/ Wormian bones
- Lambdoidal (not B; Ackermann, Rogers *et al.* 2006, Richtsmeier, McGrath 1986)
- Asterion (Ackermann, Rogers *et al.* 2006)
- Coronal (Ackermann, Rogers *et al.* 2006)
- Nasion
- Bregmatic (not B; Ackermann, Rogers *et al.* 2006; Richtsmeier, McGrath 1986)
- Pterion (epipteric bones; Ackermann, Rogers *et al.* 2006)
- Parietal notch (Ackermann, Rogers *et al.* 2006)
Foramina
- Frontal (Richtsmeier, McGrath 1986, Berry, Searl 1963)
- Palatina minora (Richtsmeier, McGrath 1986, Berry 1963, Self, Leamy 1978)
- Maxillary foramen I (Berry 1963, Richtsmeier, McGrath 1986, Self, Leamy 1978)
- Maxillary foramen II (Berry 1963, Richtsmeier, McGrath 1986, Self, Leamy 1978)
- Postcondylar canal (Richtsmeier, McGrath 1986)
- Anterior ethmoidal foramen for anterior ethmoidal branch of nasiocilliary nerve (Richtsmeier, McGrath 1986)
- Preorbital foramen (Richtsmeier, McGrath 1986)
- Foramen Sphenoidale ventral (Richtsmeier, McGrath 1986)
- Foramen ovale, open posterior(Richtsmeier, McGrath 1986; Self, Leamy 1978)
- Fenestra flocculi (Richtsmeier, McGrath 1986)
- Mental foramen (mandible; Richtsmeier, McGrath 1986)
- Extra sutural incisive foramen (Richtsmeier, McGrath 1986)
Hyperstotic/Hypostotic
- Bridging on incisive foramen (Richtsmeier, McGrath 1986)
- Bridging of palatinum majus (Berry, Searl 1963, Richtsmeier, McGrath 1986)
- Bridging of foramen ovale (Deol 1955, Richtsmeier, McGrath 1986)
- Hypoglossal bridging (Deol 1955, Richtsmeier, McGrath 1986)
- Parted frontal bones (Richtsmeier, McGrath 1986, Truslove 1952, Self, Leamy 1978)
- Pterygoid process (Deol 1955, Richtsmeier, McGrath 1986)
- Wall separating ovale and alisphenoid canal (Richtsmeier, McGrath 1986)
- Frontal fontanelle (not B; Richtsmeier, McGrath 1986)
Other
- Sutural complexity

Bilaterality of features was also recorded, where relevant. The current literature highlights that not only are unusual non-metric features more likely to be observed in hybrid groups, but that the frequency of bilateral traits is especially high in hybrids (Ackermann 2010). Furthermore, loss of dentition during life and wear obscured the signature of tooth cusp number. These data are also recorded in case there may be behavioural or biological reasons as to why some strains were more prone to tooth loss than others.

Since these features were observed on uCT scans, some of the features (particularly those near delicate parts of the bone) were not clear. In these cases, they were not recorded. For greater accuracy, scoring of traits in the scans was re-scored after initial data collection. For more accurate

interpretation of the traits scored, a scoring system of visibility of each trait is shown in Table 5.8. A score of 1 denotes “highly visible”, 2 is “some-what visible” (and may not be trustworthy) and 3 is “not visible”. The traits scored as “3” will not be evaluated in the results sections. The traits scored at 2” were evaluated at conservative estimates (i.e. only clear examples were scored if deviating from the “normal” condition).

TABLE 5.8. VISIBILITY OF TRAITS

Teeth		
Supernumerary	1	
Rotated	1	
Missing M3s	1	
Broken teeth/bifurcated teeth	1	
Teeth too worn	2	Under-scored
Peg/reduced	1	
Cusps M1s<6	2	Glare in uCT obscures detail
Cusps M2s>4	2	Glare in uCT obscures detail
Cusps M3s>3	2	Glare in uCT obscures detail
Sutural fusion		
Nasal fusion	1	
Squamosal-frontal	1	
Squamosal-parietal	2	Bone thin
Post tympanic hook of squamosal-parietal fusion	2	Bone thin
Basisphenoid-basioccipital	1	
Basisphenoid-presphenoid fusion	1	
Preoptic root	3	Bone thin
Dorsal frontal fusion	1	
Occipital-pteriotic	2	Bone thin
Extra sutures		
Nasal	1	
Premaxilla	1	
Maxilla	1	
Frontal	2	Bone thin in places
Zygomatic	1	
Squamosal	3	Bone thin
Parietal	1	
Interparietal	1	
Occipital	1	
Palatine (not seeing much)	2	Bone thin in places
Basisphenoid	1	
Wormion bones		
Lamdoidal	1	

asterion	1	
coronal	1	
nasion	1	
Bregmatic	1	
Pterion (epipteric bones)	2	Bone thin in places
Parietal notch	3	Bone thin near border
Foramina		
Frontal	1	
Palatina minora	3	Bone thin
maxillary foramen I	1	
Maxillary foramen II	2	Bone thin in places
Foramen spenoidal ventrale	2	Bone thin in places
Postcondylar canal	1	
Anterior ethmoidal foramen	3	Bone thin in places
Preorbital foramen	2	Bone thin in places
Foramen Ovale	3	Bone thin in places
Fenestra flocculi	3	Bone thin in places
Mental foramen	1	
Extra sutural incisive foramen	1	
Hypostotic/ Hyperstotic		
Bridging on incisive foramen	1	
Bridging onf palatinum majus	3	Bone thin in places
Bridging of foramen ovale	3	Bone thin in places
Hypoglossal bridging	3	Bone thin in places
Parted frontal bones	1	Age of mouse might have affect
Frontal fontanelle	1	
Pterygoid process	3	Bone thin in places
Wall separating ovale and alisphenoid canal	3	Bone thin in places

Scoring For Sutural Complexity

Sutural complexity is normally studied using metric analyses (Wu, Chien *et al.* 2007, Skrzat, Walocha and Zawaliński 2004, Miura, Perlyn *et al.* 2009, Byron, Borke *et al.* 2004, Byron 2006), and greater sutural complexity has been correlated with increased muscle mass and muscular force (Byron *et al.* 2004), and an increase in osteoclastic activity (Byron 2006). However, for the purposes of this thesis, it is sufficient to look at a ranked order of complexity. Thus, in order to take into consideration the variability in morphology, a non-metric scoring system has been used for parietal complexity (Table 5.9, Figure 5.4). Furthermore, we will consider the differences between small deviations (score = 1), and singular, but dramatic deviations (score = 5) along the parietal suture.

TABLE 5.9. SCORING USED FOR PARIETAL COMPLEXITY

Score	Explanation
1	Straight
2	1-3 slight deviations
3	4-5 slight deviations, or 1-2 dramatic deviations
4	>5 slight deviations, 3 or more dramatic deviations
5	Single or double dramatic deviations-left or right, often near lambda

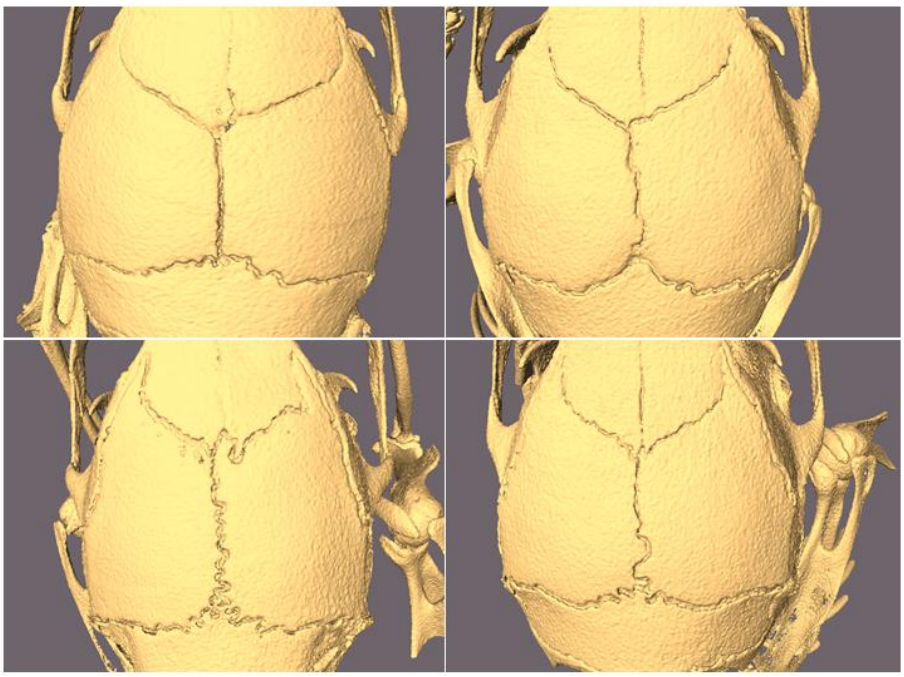

FIGURE 5.4. PARIETAL SUTURAL SCORE. CLOCKWISE FROM TOP LEFT: CAS16 (SCORE=1); CZE8 (SCORE=3); CZEXWSB31 (SCORE=5), SPR35 (SCORE =4).

Purpose: *To create continuity between research conducted on baboon and tamarin hybrids, and the research intended for the Mouse Hybrid Project. To introduce the research for the Mouse Hybrid Project, particularly research on the cranio-mandibular morphology of mouse hybrids.*

CHAPTER 6

CRANIOMANDIBULAR FORM OF FIRST GENERATION MOUSE HYBRIDS: A MODEL FOR HOMININ HYBRIDIZATION

*Submitted to the *Journal of Human Evolution*

SUMMARY OF CHAPTER

Citation: Kerryn Ashleigh Warren, Terrence Ritzman, Robyn A. Humphreys, Christopher J. Percival, Benedikt Hallgrimsson, Rebecca Rogers Ackermann. Craniomandibular form and body size variation of first generation mouse hybrids: a model for hominin hybridization. *Journal of Human Evolution*. In Review.

The overall goal of this thesis is to quantify and understand hybrid cranio-mandibular morphology in mice taxa, as models for mammalian hybrid morphologies and, ultimately, hominin hybrid morphologies. The intention of this manuscript is to create some degree of continuity between research conducted on mammalian hybrids in the past (on baboons, gorillas and tamarins), and research conducted within the larger scope of this thesis. In order to do this, the analyses employed within this manuscript are similar to those used within the above studies. This has allowed us to compare trends seen in the studies above with those of the intraspecific mouse hybrids, and whether there is consistency of morphological patterns in all three intraspecific crosses analysed.

Results show that F1 hybrids display significant cranio-mandibular size transgression, with certain measurements consistently larger in hybrids than in both parents. These measures reflect occipital length and maxillary/mandibular alveolar length. Principal component analyses of Procrustes coordinates, however, indicate that hybrids are mainly intermediate in cranio-mandibular shape compared with their parents. However, a smaller proportion of the shape variation indicates hybrid transgression (exceeding parental shapes).

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This letter is to confirm that Kerryn Ashleigh Warren was the primary author on the manuscript "Craniomandibular form and body size variation of first generation mouse hybrids: a model for hominin hybridization." This manuscript is currently in review in the Journal of Human Evolution.

Ms Warren's contributions are as follows: Collected mandibular landmarks, analysed cranial and mandibular data, performed calculations and wrote the manuscript.

Co-authors contributed as follows: RAH collected and analysed postcranial data (a minor part of the manuscript), TBR, CJP and BH helped in editing, mouse handling and interpretation of results. And I (RRA) conceptualised the project, and the Mouse Hybrid Project more broadly, and helped write the manuscript.

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ABSTRACT

Hybridization occurs in a number of mammalian lineages, including among primate taxa. Analyses of ancient genomes have shown that hybridization between our lineage and other archaic hominins in Eurasia occurred numerous times in the past. However, we still have limited empirical data on what a hybrid cranium looks like, or how to spot patterns of hybridization among fossils for which there are no genetic data. Here we use experimental mouse models to supplement previous studies of primates. We characterize size and shape variation in the cranium and mandible of three wild-derived inbred mouse strains and their first generation (F_1) hybrids. The three parent taxa in our analysis represent lineages that diverged over approximately the same period as the human/Neanderthal/Denisovan lineages and their hybrids are variably successful in the wild. Comparisons of body size, as quantified by long-bone measurements, are also presented to determine whether the identified phenotypic effects of hybridization are localized to the cranium or represent overall body size changes. The results indicate that hybrid cranial and mandibular sizes, as well as limb length, exceed that of the parent taxa in all cases. All three F_1 hybrid crosses display similar patterns of size and form variation. These results are generally consistent with earlier studies on primates and other mammals, suggesting that the effects of hybridization may be similar across very different scenarios of hybridization, including different levels of hybrid fitness. This paper serves to supplement previous studies aimed at identifying F_1 hybrids in the fossil record and to introduce further research which will explore hybrid morphologies using mice as a proxy for better understanding hybridization in the hominin fossil record.

Key words: gene flow, *Mus musculus*, introgression, heterosis, transgressive traits

1. INTRODUCTION

1.1 HYBRIDIZATION AND ITS PHENOTYPIC CONSEQUENCES IN PRIMATES

Hybridization, the interbreeding between individuals from genetically differentiated lineages, is an important mechanism facilitating evolution (Stebbins, 1959; Lewontin, 1966; Arnold, 1992; Dowling and DeMarais, 1993; Dowling and Secor, 1997; Barton, 2001; Seehausen, 2004; Schwenk et al., 2008; Arnold and Martin, 2009; Feder et al., 2012; Dittrich-Reed and Fitzpatrick, 2013; Kronforst et al., 2013; Abbott et al., 2013). While botanists have embraced hybridization as normal and abundant among diversifying taxa, it is often overlooked in studies involving animals (Mallet, 2005). Despite this, animal hybrids are quite common, with 10% of animal species producing hybrids, and with occasional "phylogenetic hotspots" having greater hybridization rates in animals than in plants (Mallet, 2005; Stelkens and Seehausen, 2009). Hybridization occurs across a wide range of mammalian lineages, including (but not limited to) whales (Árnason et al., 1991; Bérubé and Aguilar, 1998), wildebeest (Brink, 2005; Ackermann et al., 2010); bison and domestic cattle (Baranov and Zakharov, 1997); coyotes, wolves and dogs (Mahan et al., 1978; Vilà et al., 2003; Benson et al., 2012; Khosravi et al., 2013; Monzón et al., 2014); squirrels (Goodwin, 1998; Chavez et al., 2011); and many primate taxa (Jolly, 2001; Detwiler et al., 2005; Arnold and Meyer, 2006; Cortés-Ortiz et al., 2007; Zinner et al., 2011).

In primates, hybridization in the wild occurs within all major lineages. In strepsirrhines, hybridization has been reported among subspecies and species of lemurs, and especially taxa within the genus *Eulemur* (Curtis and Zaramody, 1998; Wyner et al., 2002; Pastorini et al., 2009). In platyrrhines, hybridization has been observed among howler monkeys (genus *Alouatta*; Gregorin, 2006; Aguiar et al., 2007; Aguiar et al., 2008; Kelaita and Cortes-Ortiz, 2009; Cortés-Ortiz et al., 2015), spider monkeys (genus Ateles; Rossan and Baerg, 1977), saddle-back tamarin (Saguinus fuscicollis) subspecies (Cheverud et al., 1993; Peres et al., 1996; Kohn et al., 2001), and among different species of marmoset (Coimbra-Filho et al., 1993; Tagliaro et al., 1997; Marroig et al., 2004; Malukiewicz, 2013; Fuzessy et al., 2014; Malukiewicz et al., 2014). Within Old World Monkeys, baboons (genus *Papio*), macaques (genus *Macaca*) and guenon species (genus *Cercopithecus*) exhibit inter- and intra-specific hybridization (Fooden, 1964; Wildman et al., 2004; Bergman and Beehner, 2004; Detwiler et al., 2005; Schillaci et al., 2005; Zinner et al., 2009). Hybridization among ape taxa is less common than in monkeys, no doubt in part because there are simply fewer closely related, sympatric ape

taxa. However, hybridization between siamangs and gibbons (genera *Symphalangus* and *Hylobates*, respectively) has occurred in captivity (Myers and Shafer, 1979), and other instances of hybridization have occurred between closely related species both in captivity and in the wild (Montagu, 1950; Brockelman and Srikosamatara, 1984; Marshall and Sugardjito, 1986). Within-genus hybridization among great ape species and subspecies (perhaps even between-genus hybridization) may occur (see discussion in Arnold, 2008), but evidence for it is limited and restricted to the genome (Ackermann, 2010; Ackermann, 2010; Prado-Martinez et al., 2013; but see Ackermann and Bishop, 2010). Furthermore, hybridization between distinct hominin lineages (e.g. Neanderthals, Denisovans, ancient Africa-derived people) has occurred multiple times during the Pleistocene, both outside and within Africa (Patterson et al., 2006; Green et al., 2010; Krause et al., 2010; Reich et al., 2010; Hammer et al., 2011; Reich et al., 2011; Abi-Rached et al., 2011; Meyer et al., 2012; Sankararaman et al., 2012; Sankararaman et al., 2014; Lachance et al., 2012; Wall et al., 2013; Fu et al., 2013; Fu et al., 2014; Fu et al., 2015; Fu et al., 2016; Huerta-Sánchez et al., 2014; Kim and Lohmueller, 2015; Kuhlwilm et al., 2016).

The current literature indicates that there can be considerable variation in the morphological expression of hybridization, with hybrids resembling either parent taxon, being intermediate between the parent taxa (additive outcome), or having morphologies that are extreme, or novel (Cheverud et al., 1993; Rieseberg et al., 1999; Stelkens and Seehausen, 2009; Ackermann, 2010). Heterosis or dysgenesis, positive or negative deviations from the intermediate outcome, are terms typically used to describe fitness; for morphology, larger or smaller size is a proxy for increased/decreased fitness. Although the skeletons of most primate taxa have not been examined for evidence of hybridization *per se*, wide ranges of morphological variation—especially pelage and body size variation—among primate hybrids have been observed and described (Arnold, 2008; Ackermann, 2010). The work that has been done on the skeleton of primate and other mammalian hybrids shows that hybrids are extreme in size (transgressive) and sometimes express high frequencies of novel traits relative to the parents (Ackermann et al., 2006; Ackermann, 2010; Ackermann et al., 2014). These include a prevalence of atypical traits associated with a breakdown in the coordination of early development, such as supernumerary teeth and sutural anomalies (Goodwin, 1998; Ackermann et al., 2006; Ackermann et al., 2010; Ackermann et al., 2014; Ackermann and Bishop, 2010).

Phenotypic changes in plants, resulting from large scale changes in genomic regulation due to the combining of divergent genomes, is referred to as “genomic shock” (Comai et al., 2003). Such effects (like the unusual expression of growth-related genes) have also been implicated in the unusually large size of Peromyscus (field mouse) hybrids (Duselis and Vrana, 2010).

In terms of cranio-metric analyses of hybrids, research has focused on understanding heterosis and dysgenesis (where the hybrids are significantly larger or smaller, respectively, than the additive effect- intermediate between parents- expected based on parental size). Analyses that have examined baboons, gorillas and tamarins indicate that hybrids (or purported hybrids in the case of the gorillas) are heterotic (larger than expected based on the parent species) in the majority of traits tested (Cheverud et al., 1993; Ackermann et al., 2006; Ackermann and Bishop, 2010). The statistical significance of cranial heterosis varies among primate hybrids, with tamarin hybrids exhibiting more significant heterotic cranial traits than baboon hybrids. It is not known how many generations cranial heterosis persists after hybridization has taken place, though there is some suggestion that it might be observable for a considerable amount of time (Ackermann and Bishop, 2010).

Primates are excellent models for understanding hominin hybrid morphology, but they have limitations. In particular, slow breeding time, expense, and ethical issues make experimental work with primates unfeasible. Observational data in the wild, or data collected from museum specimens can be used, but often the degree of introgression is unknown or unknowable. Moreover, collecting skeletal data on wild animals is complicated (and expensive if it involves capture and radiography/scanning). Known genealogies of hybrid primates are rare, with skeletal collections such as the Southwest National Primate Research Center (SNPRC) baboons (Ackermann et al., 2006; Ackermann et al., 2014) having limited samples beyond the first generation. Additionally, both the SNPRC collection and many museum collections are of crania only; information regarding the postcranial skeleton of hybrids is practically non-existent.

Here we present data obtained from mouse crosses that have been chosen and bred to provide a more comprehensive approach to assessing variation in the hybrid skeletal phenotype. This is the first study from an ongoing project that is examining multi-generation mouse recombinants of several closely related subspecies and two species, designed to generate large samples of mice with various degrees of introgression in the wild. The data generated will ultimately include skeletal cranial and postcranial data, as well as soft-tissue (pelage, muscle) variation, in the context of known genotypes. This paper serves as an introduction to this research, bridging the gap between previous research on the effects of primate hybridization in the skeleton, and our larger hybrid mouse research project. Specifically, we are reporting on size, form and shape variation in the crania of our sub-specific crosses of mice, and comparing these results to previous work on other taxa. Ongoing and future research with these mice will include further in-depth analyses to investigate inter- and intra- specific hybrid morphologies.

1.2 MICE AS MODEL ORGANISMS

Mice are useful model organisms for evolutionary studies (Guénet and Bonhomme, 2003; Berry and Scriven, 2005). They are widely utilized, have available complete genome sequences, can be subject to tightly controlled breeding, and are relatively inexpensive to breed due to short generation times. There is also a diverse and deep literature regarding hybridization and introgression among sub-species of *Mus musculus* (Sage et al., 1993; Boursot et al., 1993; Alibert et al., 1994; Auffray et al., 1996; Renaud et al., 2009; Teeter et al., 2010; Renaud et al., 2012) and some research considering hybridization among more divergent species of the genus *Mus* (Forejt, 1996; Montagutelli et al., 1996; Greene-Till et al., 2000; Duvaux et al., 2011). This makes them useful model organisms for addressing many questions regarding the morphology of mammalian hybrids.

This study focuses on the first generation (F_1) hybrids of three different subspecies of *Mus musculus*. Our ultimate goal is to catalogue and interrogate the effects of hybridization in these crosses to more thoroughly interpret the fossil record of human evolution (see discussion). The divergence of these *Mus musculus* subspecies occurred less than 600 thousand years ago (kya), according to scnDNA (single-copy nuclear DNA) hybridization results (She et al., 1990; Boursot et al., 1993). Wild populations of crosses between these subspecies occur under different scenarios of gene flow. Between *M. m. musculus* (which occurs across northern Eurasia, from eastern Europe to Japan) and *M. m. domesticus* (in Western Europe, around the Mediterranean), a narrow hybrid zone occurs in Europe (Bonhomme et al., 1987; Teeter et al., 2008; Bonhomme and Searle, 2012; Baird and Macholan, 2012). Male hybrids are typically infertile, following Haldane's Rule, but gene flow still occurs across these groups (Payseur et al., 2004; Teeter et al., 2008; Teeter et al., 2010; White et al., 2012). In contrast, the hybrid zone between *M. m. musculus* and *M. m. casteneus* (located in southeast Asia) is extensive, with the hybrids being so successful that a hybrid taxon, *M. m. molossinus*, has formed (Yonekawa et al., 1988; Yonekawa et al., 2012). As they are not geographically proximate, there is no natural hybrid zone between *M. m. domesticus* and *M. m. casteneus*. Thus, the three different mouse crosses represent potential models for hybridization of recent (Mid-to-Late Pleistocene) hominin lineages under three very different scenarios. In these scenarios, the spatial relationships among parent taxa in the wild and outcomes of hybridization differ, possibly as a result of differences in the adaptive success and evolutionary history of the founder strains and associated crosses.

The morphology of *M. m. domesticus* and *M. m. musculus* hybrids has been quantified from inbred laboratory strains and across the hybrid zone in Europe. Much of this research looks at fluctuating asymmetry and developmental stability across this zone and within the hybrids (Leamy, 1984; Alibert et al., 1994; Auffray et al., 1996; Debat et al., 2000). A related study of mandibular size and form in

laboratory crosses indicates that hybrids are intermediate in shape, but heterotic, and indeed, transgressive, in size (exceeding that of both parents; Renaud et al., 2009; Renaud et al., 2012). Some heterosis has also been shown in other inbred laboratory strains of mice (Chai, 1956a; Carmon, 1963; Leamy, 1982a; Leamy, 1982b; Thorpe and Leamy, 1983; Leamy and Thorpe, 1984; Leamy, 1984; Percival et al., 2016). However, the degree of heterosis in the crossing of inbred laboratory strains appears to vary widely, with the source of this variability sometimes attributed to maternal effects. This pattern is also less clear across the natural hybrid zone (Pallares et al., 2016). This may be because samples collected in natural hybrid zones include multigenerational recombinants which mostly resemble parents, as well as parent strains. However, it is also likely that the natural parental populations are not as inbred as previously studied laboratory mouse populations. In this paper, we explore the skeletal commonalities of body size and cranio-mandibular form among three hybrids from the three parent subspecies described above. We therefore intend to explore potential smilarities in heterotic expression and hybrid-parent cranio-mandibular form differences among three crosses from similarly divergent parent groups. This will then be discussed within the context of the previous research which has looked at primate hybrid crania, and be used to describe and justify further research.

2. Materials and Methods

2.1 Samples and Measurements

The sample for the current study comprises mice from three wild-derived inbred *Mus musculus* strains (*M. m. casteneus* [CAST/EiJ, hereafter CAST], *M. m. musculus* [CZECHI/EiJ, hereafter CZECHI], and *M. m. domesticus* [WSB/EiJ, hereafter WSB]), acquired from the Jackson Laboratory (www.jax.org), and their F_1 hybrids (CASTxCZECHI, CASTxWSB, and CZECHIxWSB; all crosses were reciprocal). A recent haplotype diversity study of *M. musculus* indicates that wild-derived mouse strains often show a history of hybridization between these three subspecies (Yang et al., 2011) and therefore strains were chosen specifically to minimize any history of mixed ancestry, rather than for other reasons (e.g. locality). Wild-derived strains are comparable with wild-caught populations in being more resistant to infections, in having dominant genetic alleles and phenotypes when crossed with laboratory strains, in behavior (and are therefore used as model organisms for studies in behavioral genetics), and in having a large number of polymorphisms derived from natural populations (Guénet and Bonhomme,

2003). Sample sizes are provided in Table 1. Animals were housed, bred and sacrificed at the University of Calgary in accordance with relevant federal regulations and approved animal care protocols from the University of Calgary and the University of Cape Town (AC12-0210 and 2012V56RA, respectively).

Following sacrifice, micro-computed tomography (microCT) scans were obtained in the 3D Morphometrics Centre at the University of Calgary using a Scanco vivaCT40 scanner (Scanco Medical, Brüttisellen, Switzerland) at 0.035mm voxel dimensions at 55kV and 72-145µA. Analyses of body size and cranial form were then conducted. Body size was approximated from lengths of the humerus (most proximal point on the humeral head to distal end of trochlea, following Sargis [2002]), ulna (proximal edge of olecranon process to distal edge of styloid process, following Sargis [2002]), femur (proximal edge of the greater trochanter to the distal end of the lateral condyle), and fibula (distal end of the head to the distal end of the lateral malleolus), all measured from the microCT scans using the Avizo® Fire 8.1.1 software (VSG). Craniofacial form was quantified from 21 three-dimensional landmarks (Table 2; Figure 1), which were collected using Analyze 3D (version 5.0; www.mayo.edu/bir/) from bone surfaces of micro-CT images of mouse heads. These landmarks were developed in the Hallgrimsson lab and have been used in past publications (see Percival et al., 2016). In addition, 17 mandibular landmarks were collected using Meshlab (v.1.3.2) from mesh surfaces derived from the same CT images and created with Avizo.

2.2 Analyses

Pairwise comparisons of limb lengths (*t*-tests: α-level of both p=0.05 and 0.001 are used) were made between CAST, CZECHI, and WSB (hereafter, referred to as “parent strains” or “parents”) and between CASTxCZECHI, CASTxWSB, and CZECHIxWSB (hereafter, referred to as “hybrid strains” or “hybrids”). In addition, limb lengths of each hybrid were compared to those of each of its parents, creating three parent-parent-hybrid sets—i.e., comparisons were made among the following groups: 1) CAST, CZECHI, and CASTxCZECHI; 2) CAST, WSB, and CASTxWSB; and 3) CZECHI, WSB, and CZECHIxWSB.

To investigate overall cranial and mandibular size, the landmark data were converted to 38 interlandmark distances in the cranium, and 21 in the mandible (Figure 1), which were chosen to provide complete coverage of the cranium and mandible with minimal duplication. Furthermore, these distances, particularly in the cranium, were chosen to allow for greater comparison between that of previous primate hybrid studies. Geometric means for all interlandmark distances were computed separately for the cranium and the mandible, and these geometric means were compared

among the combined sample of parents and hybrids, using *t*-tests. In addition, the geometric means were compared in each parent-parent-hybrid set.

An analysis of heterosis of overall cranial and mandibular size was also performed using the geometric means of the interlandmark distances. Specifically, the mid-parental value (MPV) was calculated for each pair of parents by computing the arithmetic average of their geometric means (as per Bruell, 1964). MPVs for each pair of parents were then compared to the 95% confidence intervals of bootstrapped distributions (9,999 permutations) of means of the respective hybrids. All statistical procedures performed using the interlandmark data were conducted using PAST version 2.17c (Hammer et al., 2001). Tests of heterosis were also performed for each of the interlandmark distances. These tests were conducted in the same way as those for overall cranial size—i.e., MPVs for each pair of parents for each interlandmark distance were computed and compared to 95% confidence intervals of a boot-strapped distributions of means from their respective hybrid.

To investigate cranial form, all interlandmark distances were compared among all groups in the sample (i.e., all three parent strains and all three hybrids) using a MANOVA and Hotelling's T-tests in PAST 2.17c (Hammer et al., 2001). Significant differences in form were further assessed by comparing all interlandmark distances among the three parent strains and among the three hybrids. In addition, comparisons were made for each parent-parent-hybrid set. These comparisons were made using pairwise *t*-tests. In order to compare our data with that of previous hybrid literature, a α-level of 0.05 is used. We acknowledge that this means we should expect 5% of our distances to display significant differences due to Type II error. A more conservative α-level of 0.001 (Bonferroni corrected p-value is 0.001 for 38 craniofacial distances, and 0.002 for 21 mandibular distances) is therefore also reported when analyzing form.

In order to better visualize shape differences between parents and hybrids, Principal Components Analyses (PCAs) for each group-set (three groups each consisting of one hybrid and its two respective parents) were generated in MorphoJ (v1.06a, Klingenberg, 2011) using the Procrustes-aligned cranial and mandibular landmark data. PCAs are used to reduce the Procrustes-aligned coordinates into components representing axes of landmark coordinate covariation, which frequently separated related genotypes in our analyses. It is important to visualize these shape changes across the coordinates which contribute to the greatest amount of shape between individuals, particularly if they are shown to separate groups.

3. RESULTS

3.1 Comparisons between parent strains

Pairwise comparisons of limb length are shown in the first section of Table 3. Among parents, WSB had the longest forelimb lengths (i.e., humerus and ulna), followed by CAST, and CZECHI, respectively. WSB had significantly longer humeri and ulnae than CZECHI. WSB also has the longest hindlimb elements (i.e., femur and fibula), followed by CZECHI and CAST, respectively. CZECHI and WSB had significantly longer femora than CAST, but no significant difference in femur length existed between WSB and CZECHI. Fibular length in WSB was significantly greater than in both CZECHI and CAST, but the differences between CAST and CZECHI were not statistically significant.

Pairwise comparisons of geometric means of the cranial interlandmark distances (Table 3) indicated significant differences among parents, among hybrids, and between hybrids and their respective parents. Specifically, among parents, WSB had the largest overall cranial size based on geometric mean. Pairwise comparisons of geometric means of the mandibular interlandmark distances are also recorded in Table 3. Among parents, WSB and CZECHI are not significantly different from each other in mandibular size, yet both differ significantly from CAST.

In analyses of cranial form, the results of the pairwise *t*-tests comparing means among all groups indicated a similar pattern—i.e., a large majority of the interlandmark distances were significantly different ($p<0.05$) in each pairwise comparison, and 17/38 were significantly different in all pairwise comparisons (11 with $p<0.001$). In comparisons among parents, 26 of the 38 interlandmark distances were significantly different in all three comparisons ($p<0.05$; 22 at $p<0.001$). In addition to the differences noted above, which vary among all groups, there are specific aspects of skull form that were found to differ among the parent strains specifically (Figure 2). It is worth noting that there are differences that are common across all groups in both the cranium and mandible, and are indicated in the figures ($p<0.05$; shown in red in Figures 2-4). In particular, these differences include the anteroposterior length and mediolateral width of the temporal foramen (formed laterally by the zygomatic and squamosal portions of the zygomatic arch and medially by the lateral portion of the squamosal and frontal bones). The anteroposterior length and, to a lesser extent, mediolateral width of the posterior portion of the frontal bone also differs among the parent strains. There are also differences among these strains in the cranial base—i.e., in the anteroposterior length and mediolateral width of the sphenoid wing and the tympanic bulla.

The results of the pairwise *t*-tests comparing means among mandibles of parents indicated a similar pattern—i.e., a large proportion of the interlandmark distances were significantly different ($p<0.05$) in each pairwise comparison. In comparisons among parents, 11 of the mandibular interlandmark distances were significantly different in all three comparisons ($p < 0.05$; 7 at $p<0.001$). Although these measurements are found throughout the mandible, they are predominantly located in the anterior

portion (Figure 2). In the mandible, parent strains differ in the superoinferior height of the coronoid and condylar process as well as in the anteroposterior length and superoinferior height of the entire mandibular corpus and incisor alveolus. Furthermore, there is no difference among parent groups in the distance between the molar tooth row and the base of the incisor crown on the superior aspect of the mandible.

3.2 Comparisons between the CASxCZE hybrid and their parent strains

Postcranially, the hybrids had significantly longer long bones than both parents. The geometric means for both the cranium and mandible were also significantly larger in hybrids compared with the parents. In comparisons of cranial form of CASTxCZECHI to its parents, 22/38 interlandmark distances were significantly different and larger than in both parents (12 at $p< 0.001$). Two distances were significantly smaller in CASTxCZECHI compared to the parents ($P<0.05$; one at $p<0.001$: NAS-IOS which describes facial width). In mandibular comparisons of CASTxCZECHI to its parents, 11/21 interlandmark distances were significantly different and larger than in both parents at $p<0.05$ (8 at 0.001). Thirty-three of the cranial MPVs (87%) were outside and below the CASTxCZECHI confidence intervals. Seventeen (81%) of the mandibular MPVs were outside the CASTxCZECHI confidence intervals (16 of these were below).

3.3 Comparisons between the CASxWSB hybrid and their parent strains

Postcranially, the hybrids again had significantly longer long bones than both parents. Among all the hybrids, CZECHIxWSB had the largest geometric means for cranial size, and was significantly larger than its parent strains. The geometric mean for the mandible was significantly different between the hybrid and CAST parent, but not between the hybrid and WSB. The similar mandibular size of WSB and CASTxWSB may be because WSB had the largest overall mandibular size based on geometric mean of the parents (followed by CZECHI, and CAST, respectively), while CASTxWSB had the smallest mandibular size of the hybrids (CZECHIxWSB had the largest). In the comparison of cranial form of CASTxWSB to its parents, 21/38 distances were significantly larger than in both parents (12 at $p<0.001$). In this comparison, two distances were significantly smaller in the CASTxWSB compared to both parents (at $p<0.05$; one at $P<0.001$: PSA-MPP, which describes palatal width). In the mandibular comparison of CASTxWSB to its parents, only three distances were significantly larger than in both parents at $p<0.05$ (none at $p<0.001$), although 17 were larger than in CAST (the smaller parent). Thirty-three of the cranial MPVs (87%) were outside and below the confidence intervals of CASTxWSB.

Seventeen (81%) of the mandibular MPVs were outside and below the confidence intervals of CASTxWSB.

3.4 Comparisons between the CZExWSB hybrid and their parent strains

Postcranially, the hybrids again had significantly longer long bones than both parents. In the comparison of cranial form of CZECHIxWSB to its parents, 22/38 of the interlandmark distances were significantly longer than in both parents at $p<0.05$ (12 at $p<0.001$). In this comparison, two of the interlandmark distances were significantly smaller in CZECHIxWSB compared to both parents (at $p<0.05$, none at $p<0.001$). In the mandibular comparison of CZECHIxWSB to its parents, 9/21 of the interlandmark distances were significantly larger than in both parents at $p<0.05$ (6 at 0.001). Thirty-five of the 38 cranial MPVs (92%) were outside and below the confidence intervals of CZECHIxWSB. Nineteen (90%) of the 21 mandibular MPVs were outside of the confidence intervals of CZECHIxWSB (17 of which were below).

3.5 Comparing the three hybrid-parent datasets to identify common trends

In all parent-parent-hybrid sets (i.e., comparisons of hybrids to their respective parents), the hybrids had significantly longer lengths than parents for all the long bones that were measured. Among the hybrids, CASTxCZECHI have longer ulnae and fibulae, with ulnar length being significantly longer than in the other hybrids. There were also significant differences in femoral length between CASTxWSB and CZECHIxWSB and between CASTxWSB and CASTxCZECHI (CASTxWSB smaller in both cases).

In the analyses of size heterosis for the geometric means of all cranial interlandmark distances, the parental MPVs fall outside and below the 95% confidence intervals of the bootstrapped distribution of hybrid means in all cases. This is expected as hybrids possessed significantly larger cranial geometric means than their respective parents in all comparisons.

For the pairwise *t*-tests, across all comparisons of hybrids to their respective parents, the results indicated that a majority of interlandmark distances in the hybrids are significantly different ($p<0.05$) from their respective parents. Figure 4 shows that in four additional measurements, the three hybrids are significantly different (at $p<0.05$) than their respective parents (in measurements associated with the basicranium these measurements are greater in hybrids than in all parents). At $p<0.001$, all three hybrids are transgressive (greater than both parents) in the length of the palate. As shown in Figure 4, there are fewer differences between hybrids and parents than there are among the parents. It should

be noted, however, that this result may be due to the fact that the patterns of differences between hybrids and parents are not identical across the three parent-parent-hybrid sets. In other words, the similarities/differences between any one hybrid and its respective parent strains may not be the same as those between another hybrid and its respective parents, resulting in a relative dearth of measurements that are different in all parent-hybrid comparisons. Despite this potential caveat, there is at least one cranial region (i.e., length of the anterior portion of the zygomatic arch) that differs uniquely between hybrids and parents.

In 13 of the total 38 measurements, all the hybrids are significantly larger than either parent (at $p<0.05$, 9 at $p<0.001$). Figure 5, which illustrates the consistent differences between each hybrid and its respective parents, demonstrates that all measurements are either heterotic and/or significantly larger in hybrids relative to parents generally and corroborates the results of comparisons of overall cranial size by demonstrating the overall larger size of each hybrid relative to its parents. The only cranial regions in which hybrids are not larger than parents are the anteroposterior length and mediolateral width of frontal bone (especially in the more posterior portion), the superoinferior height of the posterior part of the neurocranium, and the mediolateral width of the palatine bone. Only one measurement, reflecting facial width, is significantly smaller in all three hybrids relative to parents at $p<0.05$ (no differences are significant at $p<0.001$).

For the mandible, the results are similar to that seen in the cranium, with pairwise *t*-tests indicating that a majority of interlandmark distances in the hybrids are significantly different from their respective parents. Figure 4 shows that in five measurements the three hybrids are significantly different from their parents ($p<0.05$, none at $p<0.001$): in measures of the tooth row and anterior mandible especially. If we include measurements in which the hybrids were significantly different from the parents in four or five of the six potential comparisons, the measurements are seen throughout the mandible, possibly reflecting the overall mandibular heterosis. In the majority of these measurements, the hybrids are greater in size than one or both parents. None of the interlandmark distances were significantly smaller in any of the hybrids compared to both of their parents ($p<0.05$).

In analyses of cranial heterosis, MPVs for the majority of the interlandmark distances were outside and less than the 95% confidence intervals of the bootstrapped distribution of the respective hybrids. Notably, MPVs for 31 of the interlandmark distances were outside and below the confidence intervals of the respective hybrids in all parent-parent-hybrid sets. This is a strong indication that hybrids are larger than parental midpoints in the majority of the cranial measurements (Figure 5).

Similarly, in analyses of mandibular heterosis, MPVs for the majority of the interlandmark distances were outside and below than the 95% confidence intervals of the bootstrapped distribution of the respective hybrids. MPVs for 13 (62%) of the interlandmark distances were outside and below the

confidence intervals of the respective hybrids in all parent-parent-hybrid sets (Figure 5). There are also fewer differences between hybrids and parents in the mandible, and the specific differences that are unique to the hybrid-parent comparisons reside in the mandibular corpus near the molar tooth row and in the anteroposterior length and superoinferior height of incisor alveolus.

Figure 6 shows the PCAs of the Procrustes-aligned cranial landmark data. In all three analyses, PC1 (between 36.8% and 43.9% of the total variance of each PCA) indicates that the largest proportion of variance in cranial shape separates the parental groups. On plots of specimens along PC1 and PC2, the hybrids are largely intermediate, although overlapping more with one of the parents along PC1. PC1 largely reflects shape differences between parent strains, which appear to differ in relative cranial length and neurocranial height in all three PCAs. This agrees somewhat with the pattern seen in the analyses of linear distances, although the features noted in our PCA analysis are obscured by the large differences in absolute skull size noted between hybrids and parents. In each PCA, the hybrid group is more extreme along PC2 (between 11.9 and 16.4% of the total variance), with the parents overlapping greatly at the other extreme. Shape variation along PC2 is associated with differences in relative snout/palatal length, facial/anterior-temporal width, neurocranial height and, width of cranial base in all three PCAs, where hybrids appear to have relatively longer snouts, thinner faces and shorter heads. This differs from the comparisons of linear distances, possibly because distances related to snout length differed in all pairwise comparisons.

Figure 7 shows the PCAs for the Procrustes-aligned mandibular landmarks. Similar to the pattern seen in the crania, plots of PC1 and PC2, which represent the majority of the variance (PC1: between 43% and 47%), show the parental strains separated along PC1, with hybrids found intermediately. In all three crosses, the shape differences associated with PC1 and PC2 are found across many of the mandibular landmarks, but there are stronger shape differences in coronoid height and position. These are features that differed between all parents in comparisons of interlandmark distances, which are measurements of size, while these PCAs are derived from landmark coordinates after removing size. However, there are other differences unique to each parent cross such as along the molar row between CAST and WSB, and CAST and CZECHI and along the inferior border between CAST and WSB and CZECHI and WSB. Along PC2 (between 12.6% and 19% of variance), the hybrid is a clear extreme in the CASTxCZECHI cross and the CZECHIxWSB cross, but in the CASTxWSB cross there is a lot more overlap between the hybrids and parents. Variation in posterior and inferior profiles of the mandibles seem to be the shape change most prevalent in all three crosses along PC2, with differences along the molar alveolar region also prevalent. Relative molar length was a feature observed to differ between hybrids and parents in mandibular form.

4. DISCUSSION

4.1 BODY SIZE AND LIMB LENGTH

The results presented here show that the F_1 hybrid mice have longer limbs than their respective parents. This result was consistent across all parent-parent-hybrid sets. Taken together, and based on previous definitions (Chai, 1956b), these results suggest that the F_1 mouse hybrids examined here can be considered transgressive (more extreme; in this case larger than parents) in terms of the postcranial skeletal elements measured here. The results of comparisons of body size (limb length) and overall cranial size—i.e., that hybrids, by and large, have larger crania and longer limbs—are consistent with previous studies of mouse hybrids (Eaton, 1953; Chai, 1956b; Carmon, 1963; Leamy, 1982a; Thorpe and Leamy, 1983; Kurnianto et al., 1999; Pallares Amaya, 2015; Percival et al., 2016; Pallares et al., 2016). It is important to note that although these previous studies, like the present study, used strains representing subspecies of *Mus musculus*, not all strains (or even subspecies) included in the previous work are identical to those used in this study.

It is possible that this effect may be partially explained by restored heterozygosity associated with hybridization between inbred parent strains; inbreeding in the parent strains can make strains smaller on average (Bruell, 1964; but see Lynch, 1977) with hybridization returning the morphology to a natural size (Lerner, 1954; Barnett and Scott, 1964; White, 1972). This may explain why the mice used in this study are more extreme in morphology compared with the natural population hybrids (Pallares et al., 2016). However, studies with both inbred and non-inbred mice (derived from natural populations) show that hybridized (outbred) mice derived from *M. m. musculus* and *M. m. domesticus* are more extreme in both cases (Lynch, 1977; Renaud et al., 2009). The less extreme morphology seen in natural populations may be masked by sampling individuals that have extensively recombined with parents. These results (of hybrid heterosis) are also generally consistent with previous studies of primates, which have noted transgression in both size and other phenotypic traits for a number of primate hybrids (Kohn et al., 2001; Ackermann et al., 2006; Kelaita and Cortes-Ortiz, 2009; Ackermann, 2010; Ackermann et al., 2014). In the most directly comparable study, when considering postcranial morphologies of the hybrids of two tamarin crosses (*Saguinus ffuscicolis illigeri* x *S. f. lagonotus* and *S. f. illigeri* and *S. f. leucogenys*), significant heterosis was found in six and 15, out of 50, postcranial

measurements, respectively (although the hybrids were heterotic, albeit not significantly so, in 45 and 44 traits, respectively).

4.2 Craniomandibular Form

Similarly, based on the results of the analyses of cranial size, F_1 mouse hybrids have significantly larger crania than their respective parents; this finding is shared across all parent-parent-hybrid sets. Similar results are seen in the mandible, although in the case of comparisons between the smaller of the three hybrids (CASTxWSB) and its larger parent (WSB), mandibular size was not transgressive. It is worth noting that in this instance, the mandibular geometric mean of CASTxWSB was similar to the larger parent, but larger than the midparental mean, which still suggests heterosis. Hybrids between wild-derived strains observed in other studies show similar patterns (Leamy, 1982a; Thorpe and Leamy, 1983; Leamy and Thorpe, 1984; Leamy, 1992; Percival et al., 2016), with F_1 hybrids having larger overall cranial size than parent (i.e. founder) strains. Other studies that have looked at wild-derived inbred strains representing the subspecies and their hybrids in Europe have similarly shown marked heterosis in cranial, mandibular and dental metrics (Alibert et al., 1997; Debat et al., 2000; Renaud et al., 2009; Renaud et al., 2012). Studies of natural hybrid zones, however, are more ambiguous. Pallares and colleagues (Pallares et al., 2016) found that measures for craniofacial and mandibular size show a weak trend, with samples increasing in size towards the center of the hybrid zone, but that this pattern is non-significant. This contrast may be due to samples comprising a mixture of parents, F_1 hybrids and multigenerational recombinants.

This finding is also consistent with previous studies of primate crania that demonstrated increased size in hybrids (Cheverud et al., 1993; Ackermann et al., 2006; Willmore et al., 2009), although levels of cranial heterosis/transgression in primate hybrids appear variable among different species. In saddleback tamarins, two groups of hybrids from different subspecies of saddleback tamarins show a similar pattern of heterosis, although they differ in extent (Cheverud et al., 1993). *Saguinus fuscicollis illigeri* x *S. f. lagonotus* hybrids displayed cranial measures that were significantly larger than both parents for two traits (in the basicranium and palate), with 56% of measures heterotic relative to a mid-parental value, and hybrids generally displaying extreme size and size-related shape relative to parents. While these hybrids closely resembled their larger parent, lack of size additivity in this hybrid was noted. Hybrids between *S. f. illigeri* and *S. f. leucogenys* had cranial measurements that were larger than either parent for the majority of traits, hinting at heterosis as well. However, few of these differences were significant, possibly because of small sample size. For baboons, hybrids between

olive and yellow baboons appeared transgressive, or significantly larger than both parents, in one metric signifying basicranial length (Ackermann et al., 2006). The majority of measurements (78%) were larger than the mid-parental value (heterotic), although only 8% were significantly so, with no significant dysgenesis (smaller than the MPV). Thus, both the excess size in hybrid primates for many measurements, as well as transgression in the basicranium, is consistent with our results from comparisons of cranial form in hybrid and parent mice. For mice, only one measure, describing the width of the nasal bones, displayed significant dysgenesis in all three mouse crosses.

4.3 Consistency of heterosis and transgression in hybrids

Probably the most significant result of this study is that the morphological trends of large size and extreme shape, and the pattern of size/shape variation, are consistent across all three mouse hybrid groups, despite the very noticeable differences in natural hybrid zones and hybrid success among these three subspecies (Boursot et al., 1993; Bonhomme and Searle, 2012). This adds to previous work on mice and primates (where crosses were between only two taxa and not three) by showing that crosses between similarly divergent organisms, that nonetheless have very different contact scenarios, result in comparable patterns of transgression. These consistencies of form (size plus shape) include more extreme size in hybrids, as well as relatively longer snouts in the hybrids, as reflected in significant differences between them and their parents in facial and palatal measurements. Hybrids also display larger cranial base and cranial vault measures, as reflected in significant differences in measures of the petrous temporal bone and parietal bone, among other measurements.

In the PCA of Procrustes coordinates (which removes overall scale), the axis of greatest variance (PC1) separates parents, with hybrids intermediate. Hybrids are distinguished from from parents along PC2 (with the exception of the CASTxWSB mandibular analysis). The shape changes associated with hybrids along PC2 include shorter relative neurocranial height, longer relative snout length and molar rows compared with parents. These relationships are consistent with what is observed in the interlandmark distance hybrid-parent comparisons, though the latter are somewhat obscured by the inclusion of size in those comparisons.

Although other studies of the crania of mice and primates have produced similar results, there are some differences in the degree and pattern of expression of heterosis/transgression. Such variability in the extent to which hybridization produces transgressive or heterotic traits may be explained by different mechanisms. First, there may be inbreeding depression of the inbred parental genotypes of laboratory studies of mice, combined with restored heterozygosity in the hybrids. While mouse

hybrids in the wild do not show the same level of size transgression in the mandible and cranium, they do exhibit extreme size relative to the parents (Alibert et al., 1994; Macholan, 1996; Alibert et al., 1997; Renaud et al., 2009; Pallares et al., 2016). However, this has only been demonstrated in populations representing one of the crosses explored here (CZECHI-*M. m. musculus* and WSB- *M. m. domesticus*), which form a hybrid zone in Europe. Furthermore, observations on unknown-cross wild hybrid populations are complicated by the mixed nature of hybrid zones (e.g. parents, F1s, F2s, backcrosses) and are therefore not directly comparable to the results demonstrated here. Second, differences in phylogenetic distance/divergence in the parents may allow for greater accumulation of divergent underlying mechanisms determining size in the more divergent crosses, which may impede additivity expected in the offspring. This suggestion will be explored in future studies, using F_1 crosses between *Mus spretus* and *M. musculus* species combined with the subspecific crosses produced for this study.

There are myriad possible explanations for increased body and cranial size in hybrids vis-à-vis parents. At a very basic level, variation in the size of skeletal elements is due to differences in the rate, timing, and pattern of growth (Lieberman, 2011). Early in development, size differences can be established by the size of the initial cell condensations (which can differ based on the initial number of cells, the timing of initiation and termination of cell condensation, and the rate of cell division; Atchley and Hall, 1991) or by differences in regulatory genes that affect developmental patterns and/or the differentiation and regulation of cells that form the skeletal element in question (e.g., osteoblasts, osteoclasts, and chondrocytes). Later in development, size differences may arise via differences in the rate or duration of growth—i.e., larger size may be due to a longer growth period and/or a faster rate of growth. Although they were performed on different strains than those investigated here, previous studies of mice have shown that hybrids exhibit increased growth rate (Eaton, 1953; Chai, 1956a; Carmon, 1963), and numerous studies of laboratory rodents have established that hybrids have longer lifespans than parents (see (Myers, 1978) for a review). A systematic increase in growth hormone may underlie this increased growth rate. Although these issues are largely outside the scope of the current study, ongoing work by this group will address the genetic and hormonal bases of size differences between hybrids and parents.

We propose three non-mutually exclusive explanations for the general consistency of the results presented here. First, as a result of heterosis, all hybrids may possess relatively large masticatory muscles, which, in turn cause enlargement of the aspects of the cranium to which this musculature attaches, including the ventral and dorsal aspects of the facial skeleton, temporals, and parietals. Second, the consistency among these patterns may also result from constraint on certain aspects of cranial anatomy, even under the pressures of restored heterozygosity and resultant heterosis. This

could be related to the need to maintain functionality in the important sensory and mechanical systems housed in the cranium (e.g. vision, olfaction, mastication). Third, the common effects of hybridization on the cranium may be due to the fact that hybridization may perturb the normal process of cranial ontogeny, and, in particular, may modify the duration of specific post-natal ontogenetic phenomena. For example, the increased length of the facial skeleton may be explained by an increase in the duration of facial growth, which occurs later in post-natal ontogeny (e.g., compared to brain growth) and results in longer, more anteriorly projected faces. Increases in the duration of facial growth may also help explain the presence, in many primate hybrids, of supernumerary teeth—i.e., extended tooth morphogenesis could result from (or be correlated with) abnormally long periods of facial growth (Ackermann, 2007; Ackermann et al., 2014).

Large size and unusual morphologies are often explained by epigenetic effects early n development: perturbed growth hormone expression or a breakdown in chromatin integrity (Duselis and Vrana, 2010; Michalak, 2009; Wolf et al., 2014). Presently, we do not have the data necessary to test the relative veracity of each of these explanations.

4.4 Implications for Hybridization in Hominins

Despite the prevalence of gene flow among primates, the existence of hybridization among fossil hominin taxa has, until recently, been hotly debated (Jolly, 2001; Harvati, 2003; Currat and Excoffier, 2004; Reed et al., 2004; Ackermann, 2005; Harvati et al., 2005; Smith et al., 2005; Zilhão, 2006; Klein, 2008). It is only with the partial genome sequencing of Neanderthal and Denisovan autosomal DNA (Green et al., 2010; Reich et al., 2010; Reich et al., 2011) that researchers have definitively demonstrated that human ancestors leaving Africa in the Late Pleistocene interbred with other archaic species. Gene flow also occurred between anatomically modern humans and other (yet unknown or not yet sequenced) hominins (Wall et al., 2009; Hammer et al., 2011; Schlebusch et al., 2012). Clearly these ancient DNA (aDNA) analyses have improved our ability to identify past hybridization events, thereby greatly improving our understanding of the evolutionary history of our species. However, aDNA is currently limited to relatively recent lineages and is subject to the idiosyncrasies of preservation. Considering the prevalence of hybridization among contemporary primate taxa, and the genetic observations that gene flow occurred in the past for many other primate taxa, it is likely that hybridization events occurred multiple times during the evolution of our species (Jolly, 2001; Arnold, 2008; Ackermann, 2010), but remain undetectable via aDNA routes.

Empirical observations of a number of primate and other mammalian taxa have shown that there are unifying phenotypic characteristics that identify hybrids, including increased variation and non-metric traits and unusual morphologies (Ackermann et al., 2006; Ackermann, 2009; Ackermann et al., 2010; Ackermann, 2010; Ackermann and Bishop, 2010; Ackermann et al., 2014). Based on these studies, Ackermann and colleagues suggest a number of hominin specimens that may be hybrids or recombinants (Ackermann, 2010; Eichel, 2014; Eichel and Ackermann, 2016). Specimens such as Krapina 58, Skhul IV mandible and maxilla, Amud I, and Qafzeh 9 and 11 show dental anomalies that range from rotated premolars to dental crowding and reduced or enlarged dentition (Ackermann, 2010). Furthermore, these early *Homo sapiens* and Neanderthals have large nasal cavities, which may further hint at hybridization (Eichel and Ackermann, 2016). Similarly, Pestera cu Oase 2, from Romania, has large third molars outside the range of variation in contemporary samples (Tattersall and Schwartz, 1999; Soficaru et al., 2006; Rougier et al., 2007; Ackermann, 2010). One of the specimens associated with Oase 2, Oase 1, has recently been shown to have a Neanderthal ancestor between four and six generations previously, retaining around 6-9% Neanderthal DNA, and autosomal segments that are largely intact and therefore show little recombination (Fu et al., 2015).

Autosomal segments in non-Africans today suggest that interbreeding occurred between 37 and 86 kya and likely between 47-65 kya (Sankararaman et al., 2012). The date of the Pestera cu Oase mandible (circa 39-41 kya) is at the recent end of these estimates (and outside of the more restricted range). Moreover, Oase 1 is later than previous estimates of hybridization based on the genome from the Ust'Ishim femur, where autosomal mutation rates estimated Neanderthal gene flow occurred 7,000-13,000 years before the individual lived (i.e. between 50 and 60 kya; Fu et al., 2014). Oase 1 may be from a population that did not contribute DNA to future Eurasian populations; nonetheless the picture emerging from these studies and others is consistent with a scenario of repeated hybridization events, some of which were fairly recent (Ackermann et al., 2016). Moreover, despite the proposed prevalence of evidence for cultural interactions between Neanderthals and modern humans in Europe, East Asians share more Neanderthal genetic ancestry than Europeans (Meyer et al., 2012; Wall et al., 2013; Vernot and Akey, 2015). While there is evidence that selection has played a crucial role in the survival and removal of Neanderthal alleles in present-day populations (Sankararaman et al., 2014; Vernot and Akey, 2014), neither selection nor population size can adequately explain the higher Neanderthal ancestry in East Asians (Kim and Lohmueller, 2015; Vernot and Akey, 2015). Again, this is consistent with a complex history of admixture between modern humans and Neanderthals, with multiple pulses of gene flow, though it is also possible that European admixture has been masked by interbreeding with another ancestral population that is yet unknown (Vernot and Akey, 2015; Kim and Lohmueller, 2015).

Given that hybridization in the human fossil record is likely to be much more prevalent than previously realized, with its traces inaccessible through aDNA for most of our evolution, it is imperative that we continue to examine and quantify its phenotypic consequences. The hybrid mice studied here represent crosses between closely-related populations, similar to what we see for these hybridizing hominins. This is important because the morphological expression of hybridization differs depending on the phylogenetic distance between the parental groups (i.e. hybrids between closely related populations exhibit additivity and heterosis, while those between more distantly related populations can display dysgenesis or abnormalities). In this context, the results presented here suggest general trends that might be useful for identifying hybrids in the human fossil record. The most obvious and consistent hybrid F_1 phenotype is for cranial size and limb length that is greater than the larger parent, but with unusually high levels of heterosis in certain features such as molar length. If we extrapolate this pattern onto modern humans and Neanderthals, an F_1 hybrid cranium could potentially be as large as, or larger than, a Neanderthal cranium, with associated size-related shape differences, but the overall cranial morphology could still more closely resemble the smaller parent, the human, or with isolated heterotic features. This description can fit a number of fossil hominins in Europe which have previously been classified as modern humans, including Pestera cu Oase 2, which is described as having traits more associated with modern humans but with a large facial skeleton (Rougier et al., 2007). Since the cranium is associated with the known-hybrid Oase 1 mandible (Fu et al., 2015), this re-classification seems quite reasonable.

Although non-metric trait variation was not examined here (and indeed it is unlikely that dental trait variation in mice will provide a good analogue for hominins), other studies have suggested that atypical non-metric dental variation, especially if bilateral, is also indicative of F_1 and other hybrids (Ackermann et al., 2006; Ackermann, 2010; Ackermann et al., 2014). Both Oase 1 and 2 are also described as having unusual (and bilateral) non-metric traits, such as extremely large molars that become larger, and display greater occlusal surface size, distally along the tooth row (Trinkaus et al., 2003; Rougier et al., 2007; Ackermann, 2010). Rougier and colleagues (Rougier et al., 2007) noted the shared unusual traits between Oase 1 and 2, going as far as to say there is a “close affinity between them” (pg. 1169) and that the unusual mosaic of features could represent admixture between modern humans and Neanderthals (see also Ackermann, 2010).

Both Oase 1 and 2 have molars which exceed the range of variation for both Neanderthals and Upper Paleolithic modern humans. While transgression in the F_1 mice appears to be more widespread (although it is also recorded in the mandibular tooth row), it is likely that in subsequent generations of hybrids (Backcrosses and F_2s), a breakdown in the covariation of these traits could occur. This may result in transgressive traits that occur within an otherwise parental form. This breakdown in

integration and modularity has been demonstrated in the mandible of mice hybrids and several other species (Renaud et al., 2012), and will be further explored within the context of this research project in subsequent papers. Indeed, the metric evidence presented here, in combination with previous non-metric evidence, is pointing to a clearer picture of what we should expect a hybrid to look like in the human fossil record.

5. Further Research

The morphological trends seen across the three mouse hybrids are shared, despite the very noticeable differences in natural hybrid zones and hybrid success (Boursot et al., 1993; Bonhomme and Searle, 2012). This result, along with observational studies on other mammalian hybrids (Cheverud et al., 1993; Kohn et al., 2001; Ackermann et al., 2006; Kelaita and Cortes-Ortiz, 2009), provides compelling evidence that these trends can be extrapolated onto other mammalian species. However, certain questions remain that were not addressed in this analysis.

First, this analysis only applies to F_1 hybrids; it is not known whether a comparable morphological pattern will exist in multi-generational recombinants. To examine this, larger studies and systematic examinations of F_2 and backcrossed mouse samples are currently underway. Second, limited information is available about how and whether genetic and temporal divergence affects the morphology of hybrids. Researchers have suggested that larger divergence time may actually "lessen" the predominance of transgressive morphologies (Stelkens and Seehausen, 2009). Efforts are also currently underway to hybridize a more divergent mouse species of the same genus (Boursot et al., 1993; Auffray and Britton-Davidian, 2012; Bonhomme and Searle, 2012)—*Mus spretus*—with the *Mus musculus* taxa to test whether phenotypic effects are comparable in crosses between more divergent taxa. Finally, it is unclear how the homozygous (inbred) nature of the parent samples used here might have affected our results. Inbreeding depression, which has been shown to reduce fitness (litter size, survival and overall body weight), will ultimately affect the comparisons between the inbred parents and the F1 hybrids, making it difficult to deduce the extent to which the transgressive heterotic size of observed hybrids is due to the hybridization between the disparate groups, or due to the inbreeding depression within the parents (and restored heterozygosity in the hybrid offspring). Research comparing levels of heterosis/transgression between our F1 mice and F1 hybrids of wild-caught mouse populations is currently underway. These future analyses will undoubtedly provide a greater understanding of the morphological variation expressed in hybrids and hybrid zones.

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TABLES AND TABLE CAPTIONS

	Cranial	Mandibular	Hindlimb	Forelimb
CAST	49	30	30	30
CZECHI	28	30	29	26
WSB	49	29	30	30
CASTxCZECHI	50	30	31	30
CASTxWSB	50	30	36	30
CZECHIxWSB	37	29	30	30

TABLE 1. SIZES OF THE PARENT AND F_1 HYBRID SAMPLES USED IN THIS STUDY.

Abbreviation	Description
Cranial landmarks	
MSI	Midline superior incisor
AIF	Anterior margin of incisive foramen
AIZ	Anterior inferior zygomatic
PM	Point of greatest curvature on the posterior margin of the malar process
ASA	Anterior superior alveoli
PIF	Posterior incisive foramen
PSA	Postterior superior alveoli
AIA	Anterior inferior auditory bulla
PZA	Point of greatest curvature along posterior edge of zygomatic process of temporal bone
OAS	Occipital-auditory-sphenoid junction
OA	Occipital-auditory junction
MPP	Medial palatal-pterygoid junction
LNS	Anteriormost point along lateral zygomatic-frontal suture.
NAS	Nasion (Midline)
IOS	Intersection of frontal suture with orbital rim
MS	Superior margin of suture of temporal and zygomatic processes of zygomatic arch.
FTP	Frontal-temporal-parietal junction
BRG	Bregma (Midline)
LAM	Lambda (Midline)
MST	Point along occipomastoid suture
TYM	Superoposterior extremity of tympanic ring
Mandibular landmarks	
MPM	Anterior edge of alveolar process where first molar hits alveolus at the midline
IAR	Intersection of molar alveolar rim and base of coronoid process
ACC	Anterior edge of the coalescence of curve of masseteric ridge with post-symphyseal rugged area
IDP	Superior-most point on incisor alveolar rim at midline (at bone-tooth junctions)
PCP	Apex of coronoid process
ANG	Tip of mandibular angle
AMC	Anterior midline point on condyle
PMC	Posterior midline point on condyle
IDI	Inferior-most point on incisor alveolar rim at midline (at bone-tooth junction)
SIR	Superior-most point on inferior border of mandibular ramus
AMF	Anterior edge of the mental foramen
ARF	Anterior edge of the ramal fossa foramen. If two foramina, use lower foramen
ART	Apex of retromolar trigone
ABC	Anterior base of coronoid process at midline
AMF	Anterior edge of mandibular foramen
MAC	Most concave point on subcondylar incisive
MN	Mandibular notch

TABLE 2. DESCRIPTIONS AND ABBREVIATIONS FOR THE LANDMARKS USED IN THIS STUDY.

	Mean and standard deviation						*t*-test p-values								
	C	M	W	C*M	C*W	W*M	C-M	C-W	M-W	C-C*M	M-C*M	C-C*W	W-C*W	M-W*M	W-W*M
Postcrania															
Humerus	11.0±0.5	10.8±0.2	11.2±0.3	11.7±0.4	11.7±0.4	11.6±0.3	**0.008**	0.066	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Ulna	12.6±0.4	12.5±0.2	12.7±0.3	14.0±0.4	13.7±0.4	13.8±0.3	0.417	0.133	**0.004**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Femur	13.7±0.5	14.3±0.3	14.4±0.5	15.4±0.4	15.0±0.6	15.6±0.5	**<0.001**	**<0.001**	0.606	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Fibula	14.0±0.4	14.0±0.2	14.4±0.3	15.4±0.4	15.2±0.4	15.3±0.4	0.311	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Cranium															
NAS-BRG	6.4±0.18	6.3±0.14	6.6±0.22	6.3±0.17	6.5±0.19	6.8±0.2	0.106	0.661	0.766	0.414	0.334	0.115	**0.045**	**0.024**	**0.010**
BRG-LAM	3.6±0.36	3.6±0.25	4.1±0.2	4.1±0.2	4.4±0.25	4.1±0.23	0.997	0.874	0.939	0.722	0.830	0.690	0.435	0.405	0.252
MSI-NAS	7.1±0.27	7.7±0.14	7.4±0.26	7.9±0.22	7.8±0.25	8.1±0.28	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**
MSI-LNS	5.7±0.21	6±0.12	6.1±0.2	6.3±0.23	6.4±0.24	6.6±0.21	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.024**	**0.038**	0.090	0.125
MSI-AIF	2.2±0.09	2.5±0.08	2.4±0.09	2.4±0.07	2.3±0.08	2.6±0.09	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
AIF-PIF	5±0.22	5.4±0.09	5.2±0.14	5.8±0.15	5.7±0.13	5.9±0.16	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
AIF-AIZ	3.8±0.22	3.7±0.11	4.1±0.16	4.1±0.19	4.3±0.17	4.3±0.17	0.067	0.115	0.125	0.087	0.056	0.060	0.111	0.171	0.205
AIZ-PM	2.1±0.12	2.2±0.07	2.4±0.12	2.4±0.13	2.4±0.11	2.5±0.15	**0.015**	**0.018**	**0.018**	**0.026**	**0.043**	0.087	0.086	0.142	0.244
AIZ-ASA	2.9±0.21	3.3±0.09	3.4±0.17	3.4±0.17	3.4±0.14	3.5±0.18	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PM-PZA	6.4±0.19	6.5±0.13	6.4±0.14	6.9±0.17	6.8±0.15	6.8±0.17	0.211	0.171	0.197	0.232	0.289	0.235	0.200	0.248	0.319
PM-IOS	3.5±0.15	3.4±0.09	3.5±0.13	3.7±0.11	3.6±0.09	3.6±0.14	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
ASA-PSA	4±0.13	3.7±0.09	3.8±0.09	4.2±0.12	4.2±0.11	4.1±0.1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
ASA-PIF	1.7±0.08	1.9±0.08	1.8±0.06	1.9±0.06	1.9±0.09	2±0.07	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PSA-MPP	1.2±0.12	0.9±0.09	1.1±0.07	1±0.12	1.1±0.08	1±0.09	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PSA-FTP	5.5±0.17	5.8±0.09	6±0.18	5.9±0.12	6±0.12	6.2±0.15	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PSA-PZA	4.5±0.15	4.6±0.08	4.5±0.12	4.9±0.13	4.8±0.12	4.9±0.14	**0.014**	**0.021**	**0.026**	**0.042**	**0.047**	**0.042**	**0.043**	0.055	0.059
AIA-OA	3.4±0.1	3.4±0.09	3.7±0.08	3.6±0.09	3.7±0.08	3.7±0.1	0.417	0.515	0.816	0.836	0.932	0.907	0.770	0.559	0.479
AIA-OAS	2.9±0.09	3±0.05	3.1±0.08	3±0.08	3.1±0.09	3.1±0.07	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.003**	**0.009**	**0.012**	**0.049**	0.092
AIA-PZA	3.1±0.13	3.1±0.08	3.2±0.12	3.4±0.09	3.4±0.1	3.4±0.12	0.202	0.116	0.063	**0.026**	**0.009**	**0.003**	**0.002**	**0.001**	**0.001**
PZA-LAM	8±0.19	7.6±0.16	8.3±0.16	8.1±0.14	8.4±0.18	8.3±0.19	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PZA-BRG	7.1±0.15	6.6±0.11	7±0.14	6.9±0.11	7.1±0.12	7±0.15	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

PZA-FTP	4.7±0.2	4.8±0.15	5.1±0.14	5±0.14	5±0.14	5.1±0.18	0.521	0.313	0.177	0.138	0.073	**0.049**	**0.024**	**0.012**	**0.011**
PZA-MS	4.5±0.18	4.9±0.2	4.8±0.2	5.1±0.19	5±0.14	5.1±0.2	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.003**	**0.021**	0.076	0.235
OAS-OA	2.9±0.18	2.9±0.12	2.9±0.14	3±0.15	3±0.12	2.9±0.14	**0.037**	**0.037**	**0.046**	0.055	0.050	**0.027**	**0.028**	**0.033**	**0.016**
LNS-IOS	0.8±0.12	1±0.1	0.8±0.1	1.1±0.14	0.9±0.11	0.9±0.11	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
NAS-FTP	5.1±0.18	4.7±0.15	4.9±0.13	5.1±0.14	5.1±0.12	5.1±0.13	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
MS-FTP	5.2±0.25	5.4±0.12	5.6±0.23	5.7±0.17	5.5±0.16	5.8±0.19	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.002**	**0.006**
FTP-BRG	3.9±0.16	3.5±0.12	3.7±0.19	3.3±0.09	3.7±0.09	3.6±0.11	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.018**	0.116	0.342	0.759
LAM-MST	5.7±0.16	5.5±0.09	6.1±0.14	5.8±0.12	5.9±0.1	6±0.13	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
MST-TYM	1.6±0.13	1.5±0.08	1.6±0.12	1.6±0.09	1.6±0.1	1.6±0.1	**<0.001**	**0.026**	0.129	0.525	0.934	0.930	0.531	0.264	0.138
AIF-ASA	3.9±0.24	4.4±0.1	4.3±0.15	4.7±0.17	4.6±0.12	4.8±0.15	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PIF-MPP	2.3±0.17	2.3±0.16	2.4±0.12	2.4±0.12	2.5±0.13	2.6±0.12	0.793	0.939	0.908	0.938	0.872	0.714	0.511	0.567	0.606
MPP-AIA	5.7±0.33	5.5±0.18	5.7±0.21	6.1±0.26	5.9±0.22	6±0.21	**0.049**	**0.036**	**0.023**	**0.014**	**0.006**	**0.002**	**0.001**	**<0.001**	**<0.001**
MPP-OAS	5.2±0.3	5.1±0.18	5.5±0.17	5.7±0.22	5.6±0.2	5.6±0.18	0.086	0.067	0.052	**0.046**	**0.020**	**0.010**	**0.008**	**0.004**	**0.003**
LNS-AIF	5.5±0.25	5.4±0.1	5.8±0.17	5.9±0.23	6±0.18	6.1±0.17	0.437	0.314	0.229	0.103	**0.032**	**0.017**	**0.009**	**0.003**	**0.001**
NAS-IOS	2.3±0.1	2.4±0.14	2.4±0.11	2.3±0.12	2.3±0.1	2.4±0.09	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.004**	**0.013**
IOS-MS	4.6±0.26	4.1±0.24	4.3±0.24	4.6±0.18	4.5±0.15	4.3±0.18	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.003**	**0.005**
MST-PZA	5.5±0.22	5.4±0.18	5.6±0.16	5.9±0.14	5.8±0.19	5.9±0.17	0.851	0.888	0.937	0.829	0.742	0.421	0.490	0.550	0.496
GM	3.7±0.11	3.7±0.05	3.8±0.08	3.9±0.08	3.9±0.07	4.0±0.09	0.417	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Mandible															
MPM-IAR	2.1±0.15	2.1±0.13	2.1±0.15	2.1±0.14	2.2±0.14	2.2±0.13	**0.025**	0.961	**0.020**	0.793	**0.033**	0.531	0.558	**0.001**	0.420
MPM-ART	5.3±0.14	5.6±0.14	5.9±0.22	6.1±0.28	6.1±0.19	6.1±0.21	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.037**	**<0.001**	**0.033**
MPM-AMF	1.2±0.11	2±0.13	1.8±0.14	1.6±0.11	1.6±0.13	1.8±0.17	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.475
IAR-ABC	1.7±0.17	1.8±0.1	1.9±0.17	2±0.16	1.9±0.17	2±0.15	**<0.001**	**<0.001**	0.333	**<0.001**	**<0.001**	**<0.001**	0.179	**0.001**	0.051
IAR-ART	1.9±0.1	2±0.18	2±0.12	2.1±0.12	2±0.15	2.2±0.11	**0.029**	**0.009**	0.898	**<0.001**	**0.002**	**0.009**	0.780	**<0.001**	**<0.001**
ABC-PCP	1.6±0.15	2.2±0.12	2.3±0.15	2.3±0.2	2.2±0.19	2.3±0.16	**<0.001**	**<0.001**	**0.021**	**<0.001**	0.075	**<0.001**	0.476	**0.002**	0.393
PCP-MN	0.8±0.08	1.1±0.08	1.1±0.08	1.1±0.1	1.1±0.1	1.1±0.08	**<0.001**	**<0.001**	0.158	**<0.001**	0.979	**<0.001**	**0.027**	0.239	**0.010**
AMC-MN	1.4±0.13	1.4±0.16	1.8±0.19	1.8±0.14	1.9±0.12	1.7±0.16	0.490	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.252	**<0.001**	**0.042**
AMC-PMC	1.5±0.12	1.8±0.16	1.5±0.13	1.5±0.14	1.4±0.11	1.7±0.14	**<0.001**	0.277	**<0.001**	0.085	**<0.001**	0.497	0.077	0.281	**<0.001**
PMC-MAC	2.2±0.12	2.5±0.12	2.8±0.18	2.9±0.17	2.8±0.26	3.1±0.15	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.163	**<0.001**	**<0.001**

MN-AMF	1.9±0.1	2.1±0.09	2.1±0.12	2.2±0.11	2.2±0.1	2.2±0.11	**<0.001**	**<0.001**	0.059	**<0.001**	**<0.001**	**<0.001**	0.090	**<0.001**	**<0.001**
MN-MAC	0.8±0.1	1.1±0.08	1.1±0.11	1.1±0.13	1±0.12	1.2±0.08	**<0.001**	**<0.001**	0.067	**<0.001**	0.718	**<0.001**	**0.008**	**<0.001**	**<0.001**
MAC-ANG	2±0.13	2.3±0.17	2.3±0.15	2.2±0.15	2.3±0.19	2.4±0.15	**<0.001**	**<0.001**	0.696	**<0.001**	0.515	**<0.001**	0.473	**0.029**	0.056
ANG-ARF	3±0.16	3.4±0.13	3.6±0.21	3.6±0.2	3.4±0.21	3.9±0.16	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.005**	**<0.001**	**<0.001**
ARF-SIR	1.1±0.19	1.4±0.17	1±0.18	1.5±0.18	1.1±0.3	1.3±0.2	**<0.001**	**0.018**	**<0.001**	**<0.001**	**<0.001**	0.918	0.066	0.287	**<0.001**
SIR-ACC	2.7±0.17	2.3±0.18	2.5±0.19	2.5±0.19	2.7±0.29	2.4±0.16	**<0.001**	**<0.001**	**0.019**	**<0.001**	**0.015**	0.188	**<0.001**	0.573	0.055
ACC-IDI	2.9±0.14	3.1±0.13	3.6±0.12	3.2±0.15	3.4±0.13	3.5±0.11	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.021**	**<0.001**	**<0.001**	**<0.001**	**0.015**
IDI-IDP	1.9±0.1	2.4±0.06	2.2±0.09	2.4±0.1	2.2±0.09	2.4±0.07	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.030**	**<0.001**	0.720	**0.015**	**<0.001**
IDP-AMF	2.6±0.13	2.6±0.16	2.7±0.13	2.9±0.14	2.8±0.16	2.9±0.19	0.056	**<0.001**	**0.029**	**<0.001**	**<0.001**	**<0.001**	**0.024**	**<0.001**	**<0.001**
ACC-AMF	2±0.12	2.6±0.14	2.7±0.16	2.5±0.16	2.5±0.17	2.8±0.18	**<0.001**	**<0.001**	**0.001**	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**	**0.018**
SIR-AMF	3.9±0.27	4.3±0.24	4.5±0.29	4.1±0.26	4.6±0.37	4.4±0.33	**<0.001**	**<0.001**	**<0.001**	**0.033**	**0.010**	**<0.001**	0.650	0.166	0.057
GM	1.8±0.05	2.1±0.03	2.2±0.06	2.2±0.07	2.1±0.06	2.3±0.05	**<0.001**	**<0.001**	0.339	**<0.001**	**<0.001**	**<0.001**	0.401	**<0.001**	**<0.001**

Table 3. Means and standard deviations for cranial, mandibular and postcranial measures (mm). Results of *t*-tests comparing means for cranial and mandibular measurements and limb lengths (postcrania) among parents, among hybrids, and between hybrids and their respective parents. Bolded values are significant at $p \leq 0.05$ and underlined at $p \leq 0.001$.

FIGURES

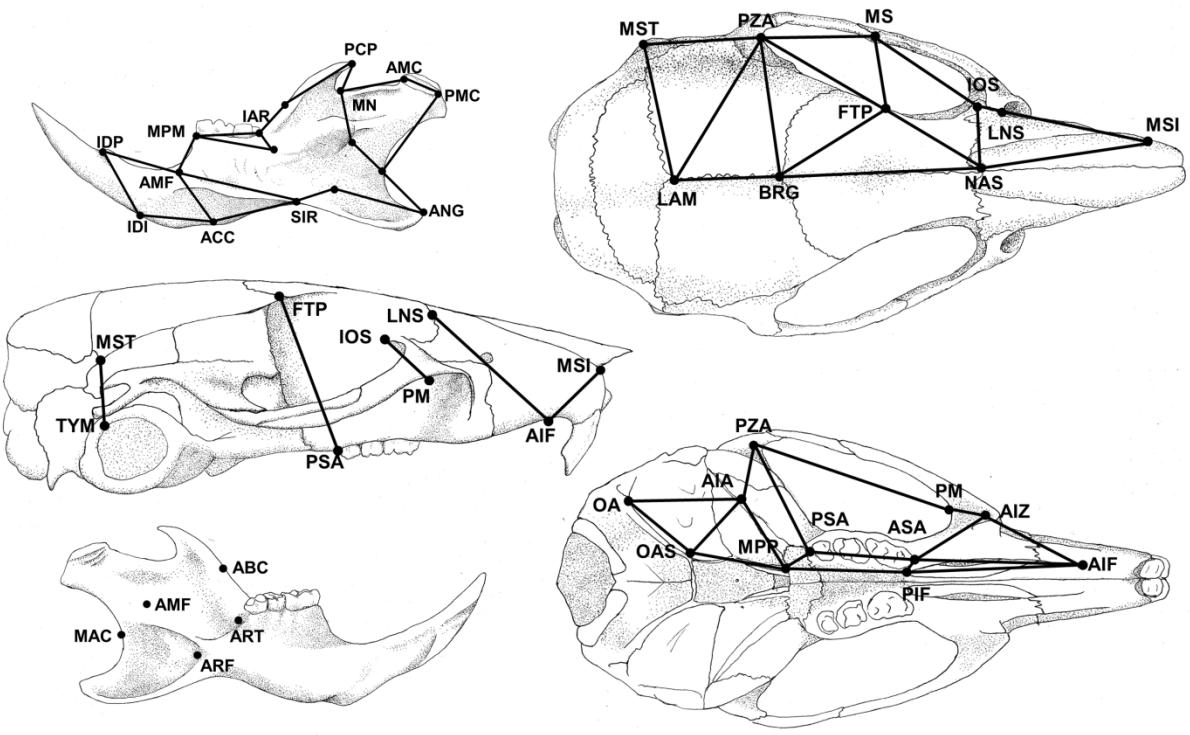


FIGURE 1: DIAGRAMS ILLUSTRATING LANDMARKS CAPTURED AND MEASUREMENTS CALCULATED IN THE CRANIUM AND MANDIBLE.

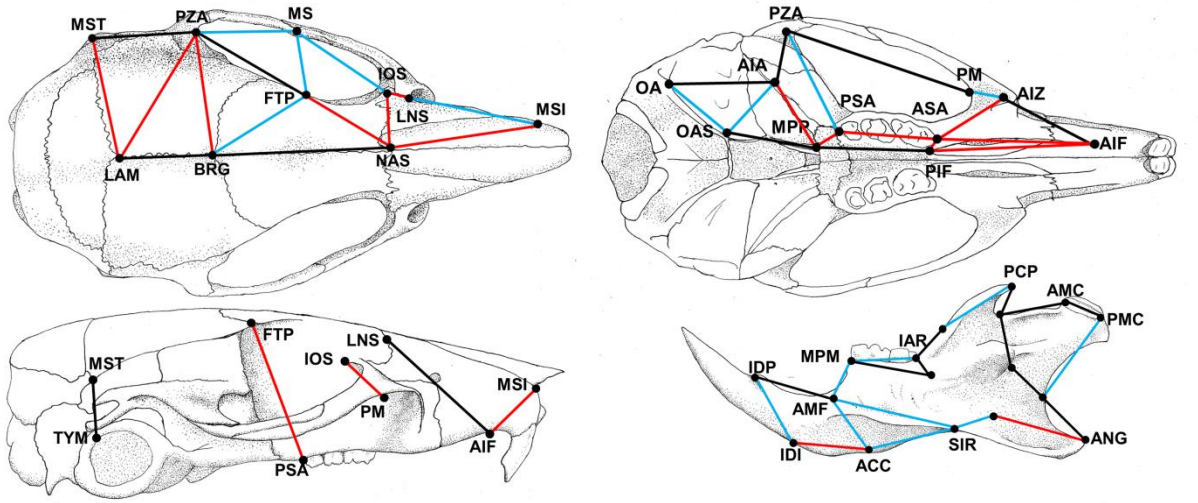


FIGURE 2: DIAGRAMS ILLUSTRATING THE RESULTS OF PAIRWISE SIGNIFICANCE TESTING FOR EACH MEASUREMENT ON THE CRANIUM AND MANDIBLE BETWEEN PARENTAL STRAINS. IN FIGURES 2-4, RED INTERLANDMARK DISTANCES ARE SIGNIFICANTLY DIFFERENT IN ALL 12 PARENTAL AND HYBRID COMPARISONS COMPARISONS AT P<0.05 (THEREFORE ALL GROUPS DEMONSTRATE SIGNIFICANT DIFFERENCES FROM ONE ANOTHER IN THOSE MEASUREMENTS). MEASUREMENTS IN BLUE IN THIS FIGURE ARE SIGNIFICANTLY DIFFERENT IN ALL THREE PARENT-PARENT COMPARISONS AT P<0.05.

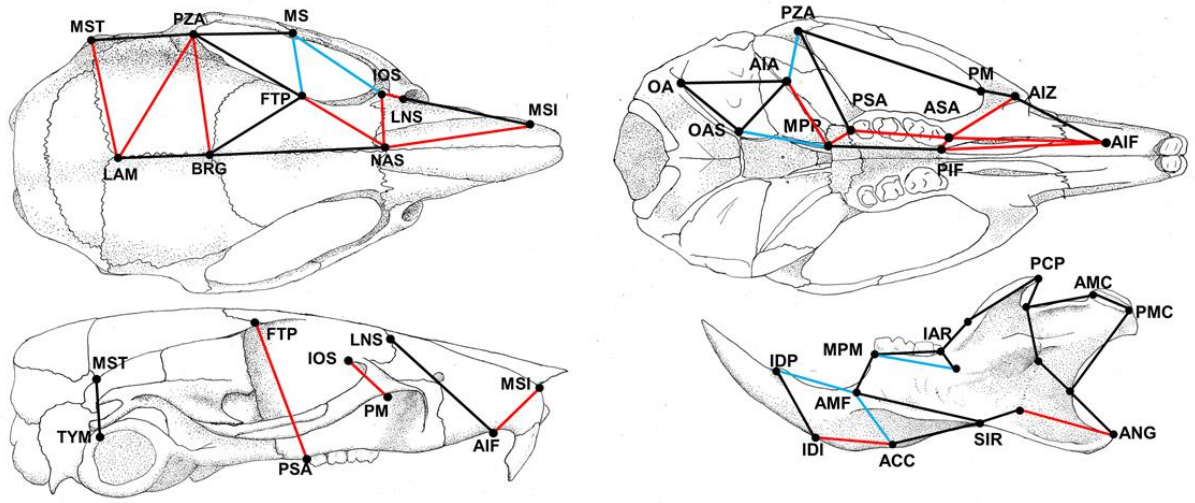


FIGURE 3: DIAGRAMS ILLUSTRATING THE RESULTS OF PAIRWISE SIGNIFICANCE TESTING FOR EACH MEASUREMENT ON THE CRANIUM AND MANDIBLE FOR HYBRID-PARENT COMPARISONS. IN FIGURES 2-4, RED INTERLANDMARK DISTANCES ARE SIGNIFICANTLY DIFFERENT IN ALL 12 PARENTAL AND HYBRID COMPARISONS COMPARISONS AT P<0.05 (THEREFORE ALL GROUPS DEMONSTRATE SIGNIFICANT DIFFERENCES FROM ONE ANOTHER IN THOSE MEASUREMENTS). MEASUREMENTS IN BLUE ARE SIGNIFICANTLY DIFFERENT IN ALL SIX HYBRID-PARENT COMPARISONS AT P<0.05.

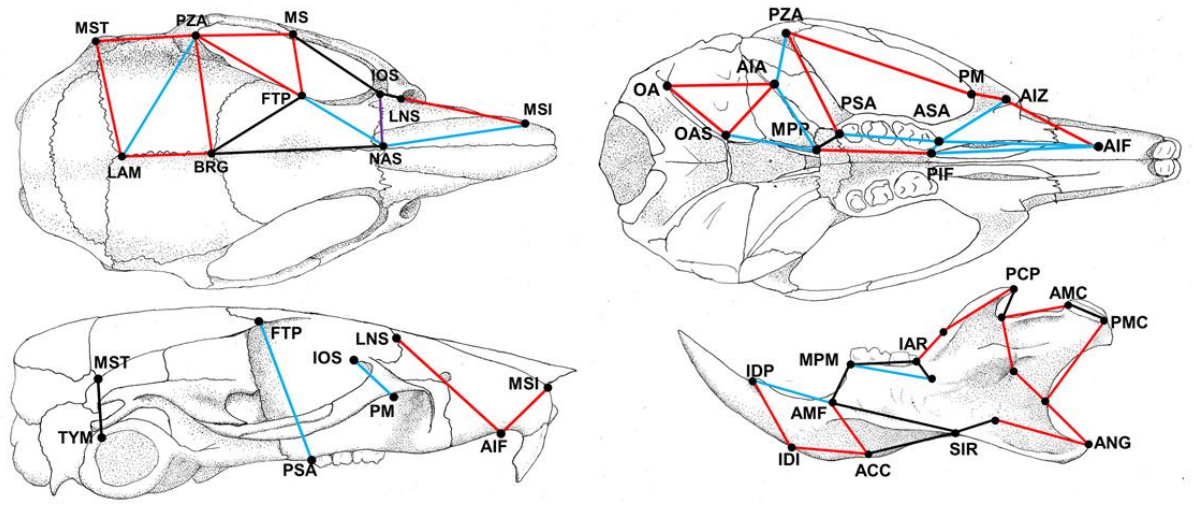


FIGURE 4: FIGURE ILLUSTRATING SIGNIFICANT HETEROSIS AND TRANSGRESSIVE MEASUREMENTS IN THE CRANIUM AND MANDIBLE. MEASUREMENTS WHERE MEAN PARENTAL VALUE (MPV) IS BELOW 95%CI OF HYBRID MEANS ARE IN RED (I.E. HETEROTIC). MEASUREMENTS WHICH ARE SIGNIFICANTLY LARGER (P<0.05) IN ALL THREE HYBRIDS RELATIVE TO THEIR PARENTS ARE PARENTS IN BLUE (I.E. TRANSGRESSIVE). MEASUREMENT WHICH ARE SIGNIFICANTLY SMALLER (P<0.05) IN ALL THREE HYBRIDS RELATIVE TO THEIR PARENTS ARE IN PURPLE (I.E. DYSGENIC).

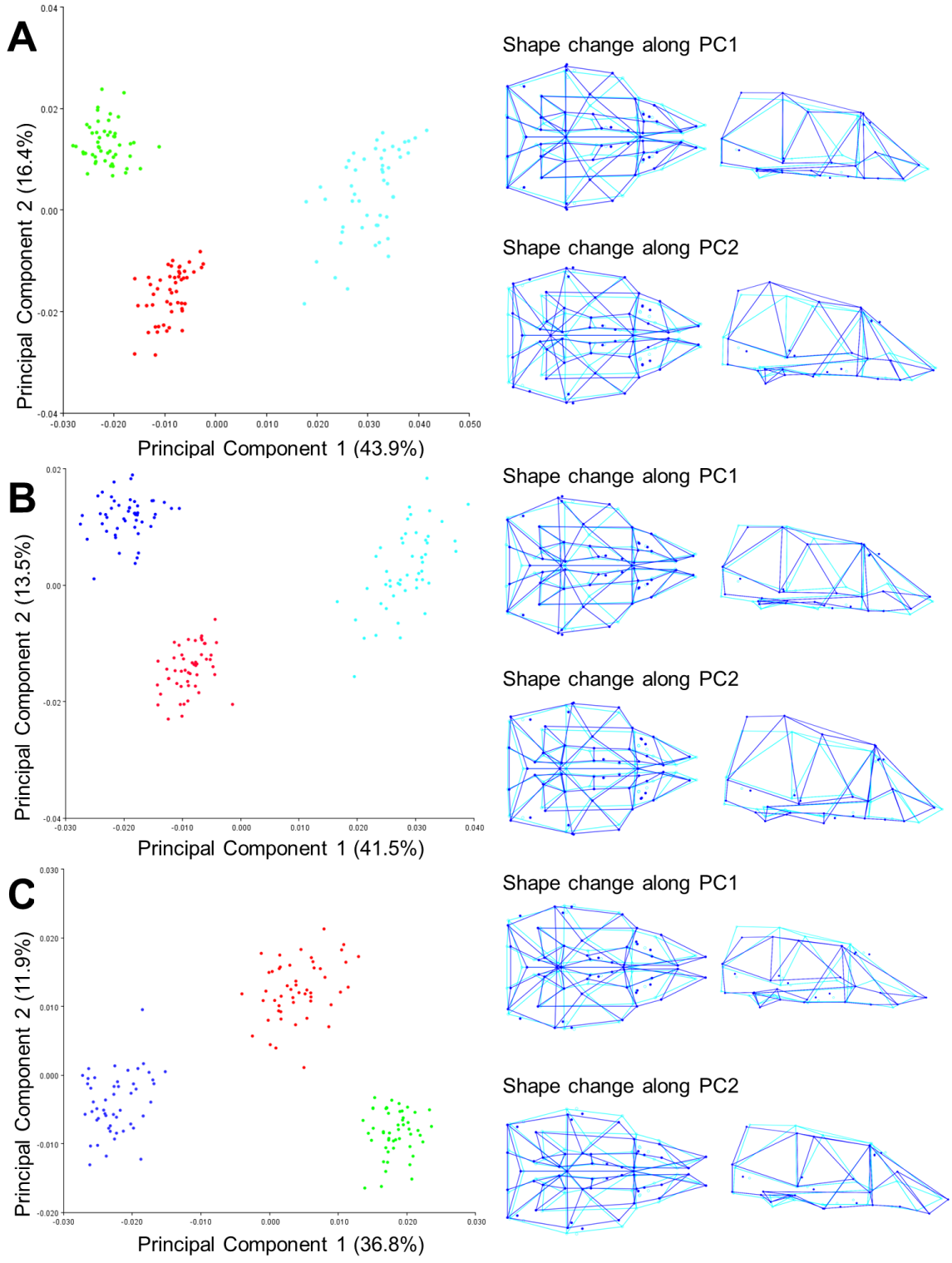


FIGURE 5: PRINCIPAL COMPONENTS ANALYSES ON THE PROCRUSTES-ALIGNED CRANIAL LANDMARKS FOR THE A) CASTXCZECHI CROSS; B) CASTXWSB CROSS; AND C) CZECHIXWSB CROSS. SPECIMENS ARE PLOTTED ALONG PC1 AND PC2, WHERE CAST SPECIMENS ARE LIGHT BLUE, CZECHI ARE GREEN, WSB ARE DARK BLUE AND F1 HYBRIDS ARE IN RED. WIREFRAME DIAGRAMS, SHOWING SHAPE CHANGE ALONG PC1 AND PC2, ARE ON THE RIGHT OF EACH PC PLOT.

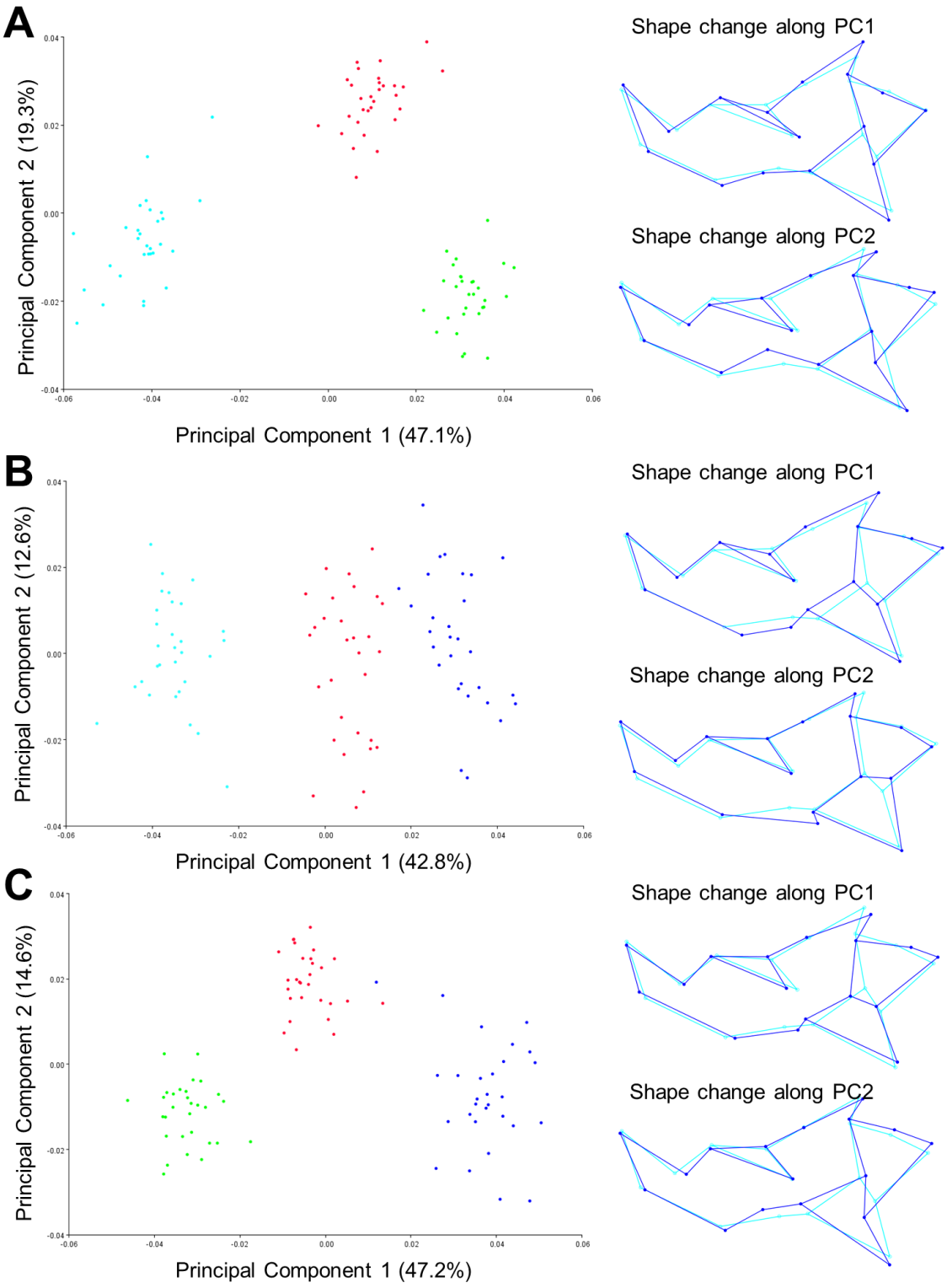


FIGURE 6: PRINCIPAL COMPONENTS ANALYSES ON THE PROCRUSTES-ALIGNED MANDIBULAR LANDMARKS FOR THE A) CASTXCZECHI CROSS; B) CASTXWSB CROSS; AND C) CZECHIXWSB CROSS. SPECIMENS ARE PLOTTED ALONG PC1 AND PC2, WHERE CAST SPECIMENS ARE LIGHT BLUE, CZECHI ARE GREEN, WSB ARE DARK BLUE AND F1 HYBRIDS ARE IN RED. WIREFRAME DIAGRAMS, SHOWING SHAPE CHANGE ALONG PC1 AND PC2, ARE ON THE RIGHT OF EACH PC PLOT.

Purpose: *To explore morphological variation in mouse hybrids and their recombinants. This includes analysing the following questions: 1) What patterns do we see with respect to geometric morphology when comparing parents, F1 hybrids and multigenerational recombinants (B1s and F2s)? 2) Are "hybrid morphologies" (large size and intermediate form/shape) retained in subsequent generations and can they be used to detect hybridization within wild mouse (and potentially other mammalian) populations where this phenomenon is unknown? 3) Are differences in morphology/variation between hybrids and purebreds the result of a breakdown in integration in cranio-mandibular elements? 4) Are the morphological patterns seen in intraspecific F1 hybrids comparable to those of interspecific hybrids?*

CHAPTER 7

MULTIGENERATIONAL RECOMBINANTS AND THE EFFECTS OF DIFFERENCES IN PHYLOGENETIC DISTANCES ON HYBRID MORPHOLOGIES

PART 1: SIZE ANALYSES

CENTROID SIZE

Figure 7.1.1 shows the box plots of cranial and mandibular centroid size for each intra-specific group (as in the methodology, group will refer to collection of parents and hybrids). The parents are in light blue (CAST), dark blue (WSB) and green (CZECHI), the F1 hybrids are in red, the F2s in orange and the B1s in pink. This colour scheme is consistent in all graphics in this chapter. There are similar patterns seen in all six charts. Parents (blues and green) are smaller than F1 hybrids (red) in both median and

mean (solid diamond) in both the mandible and the cranium. The multigenerational recombinants (B1s and F2s) are larger than the parent strains in the cranium or overlapping with the larger parent. In the mandible, the multigenerational recombinants are larger than at least one of the parents. The F1s are larger than the F2s, which are larger than the B1s in both cranial and mandibular size.

ANOVA tests on the mandible and cranium (Table 7.1.1) indicate significant differences (P< 0.0001) among hybrids and parents for all three groups in both cranial and mandibular size. Levene's tests on each group for the mandibular centroid sizes do not indicate significant inequality of variances between hybrids and parents, although the distributions of means are larger in subsequent generations (F2s and B1s) than in parents. Unlike in the mandibles, the Levene's test for homogeneity in the crania is significantly different (p<0.01) in all three comparisons. The F1 hybrids also appear to have a smaller amount of variance in size, compared to both the multigenerational recombinants and to the parents, particularly CAST.

TABLE 7.1.1 ANOVA AND LEVENE'S TESTS RESULTS FOR MANDIBULAR (LEFT) AND CRANIAL (RIGHT) CENTROID SIZE.

CAS/CZE group											
ANOVA	Df	Sum sq	Mean sq	F value	Pr(>F)	**ANOVA**	Df	Sum sq	Mean sq	F value	Pr(>F)
Strain	4	57.93	14.484	115.8	<2e-16	Strain	4	240766	60191	75.69	<2e-16
Resid	120	15.01	0.125			Resid	204	162228	795		
Levene	Df	F value	Pr(>F)			**Levene**	Df	F value	Pr(>F)		
	4	2.008	0.098				4	5.739	0.000214		
CAS/WSB group											
ANOVA	Df	Sum sq	Mean sq	F value	Pr(>F)	**ANOVA**	Df	Sum sq	Mean sq	F value	Pr(>F)
Strain	4	57.63	14.408	85.78	<2e-16	Strain	4	152534	38134	43.95	<2e-16
Resid	143	24.02	0.168			Resid	205	177872	868		
Levene	Df	F value	Pr(>F)			**Levene**	Df	F value	Pr(>F)		
	4	1.972	0.102				4	3.4918	0.008773		
CZE/WSB group											
ANOVA	Df	Sum sq	Mean sq	F value	Pr(>F)	**ANOVA**	Df	Sum sq	Mean sq	F value	Pr(>F)
Strain	3	10.32	3.439	33.83	<2e-14	Strain	2	227023	113511	163.9	<2e-16
Resid	104	10.57	0.102			Resid	147	101832	693		
Levene	Df	F value	Pr(>F)			**Levene**	Df	F value	Pr(>F)		
	3	2.024	0.115				2	7.7637	0.000623		

Tukey tests (Table 7.1.2) indicate the level of difference between group means. The F2s and B1s have smaller mandibles, on average, than the F1 hybrids but are larger than the smaller of the two parents. In the CZE/WSB group, the B1 hybrid has larger mandibular size on average than both parent strains.

All hybrids and recombinants have significantly larger mandibles than the smaller parent (where p<0.001). The CAS/WSB F1 and F2 hybrids, despite the F1 having a larger mean mandibular centroid size, are not significantly different from the larger parent (WSB).

In each group, the F1 hybrids are far greater in cranial size than any of the other strains (significantly at p<0.001). The F2s are not as large as the F1 hybrids, but are still significantly larger than the parents (at p<0.05). It is worth noting that, despite the form differences seen in the cranium (see Chapter 6), there are no significant differences in cranial size between CAST and CZECHI, which are smaller than the larger parent, WSB (which is still significantly smaller in cranial size than the F1 hybrids). The backcrossed hybrids are significantly smaller than the F1 hybrids (p<0.0001).

TABLE 7.1.2 TUKEY TEST RESULTS FOR MANDIBULAR (LEFT) AND CRANIAL (RIGHT) CENTROID SIZE (IF P< 0.05 UNDERLINED, IF P<0.001 THEN ITALICS, IF P<0.0001 THEN BOLD).

CAS/CZE group									
	CAST	**CZECHI**	**F1**	**F2**		**CAST**	**CZECHI**	**F1**	**F2**
CZECHI	**1.409**	–			**CZECHI**	-1.1	–		
F1	**1.887**	**-0.478**	–		**F1**	**80.8**	**-81.9**	–	
F2	**1.103**	0.307	**-0.784**	–	**F2**	*29*	**-30.1**	**-51.8**	–
B1	**0.911**	0.498	**-0.976**	0.192	**B1**	-1.3	0.3	**-82.1**	*30.4*
CAS/WSB group									
	CAST	**WSB**	**F1**	**F2**		**CAST**	**WSB**	**F1**	**F2**
WSB	**1.522**	–			**WSB**	**32.6**	–		
F1	**1.765**	-0.243	–		**F1**	**75.7**	**-43.2**	–	
F2	**1.416**	0.106	-0.349	–	**F2**	**53.4**	-20.8	-22.4	–
B1	**1.015**	**0.507**	**-0.75**	*0.401*	**B1**	**44.3**	-11.7	**-31.4**	9
CZE/WSB group									
	CZECHI	**WSB**	**F1**			**CZECHI**	**WSB**	**F1**	
WSB	0.113	–			**WSB**	**33.6**	–		
F1	**0.769**	**0.656**	–		**F1**	**94**	**60.4**	–	
B1	*0.411947*	*0.299*	*-0.357*		**B1**	**31.1**	-2.6	**-62.9**	

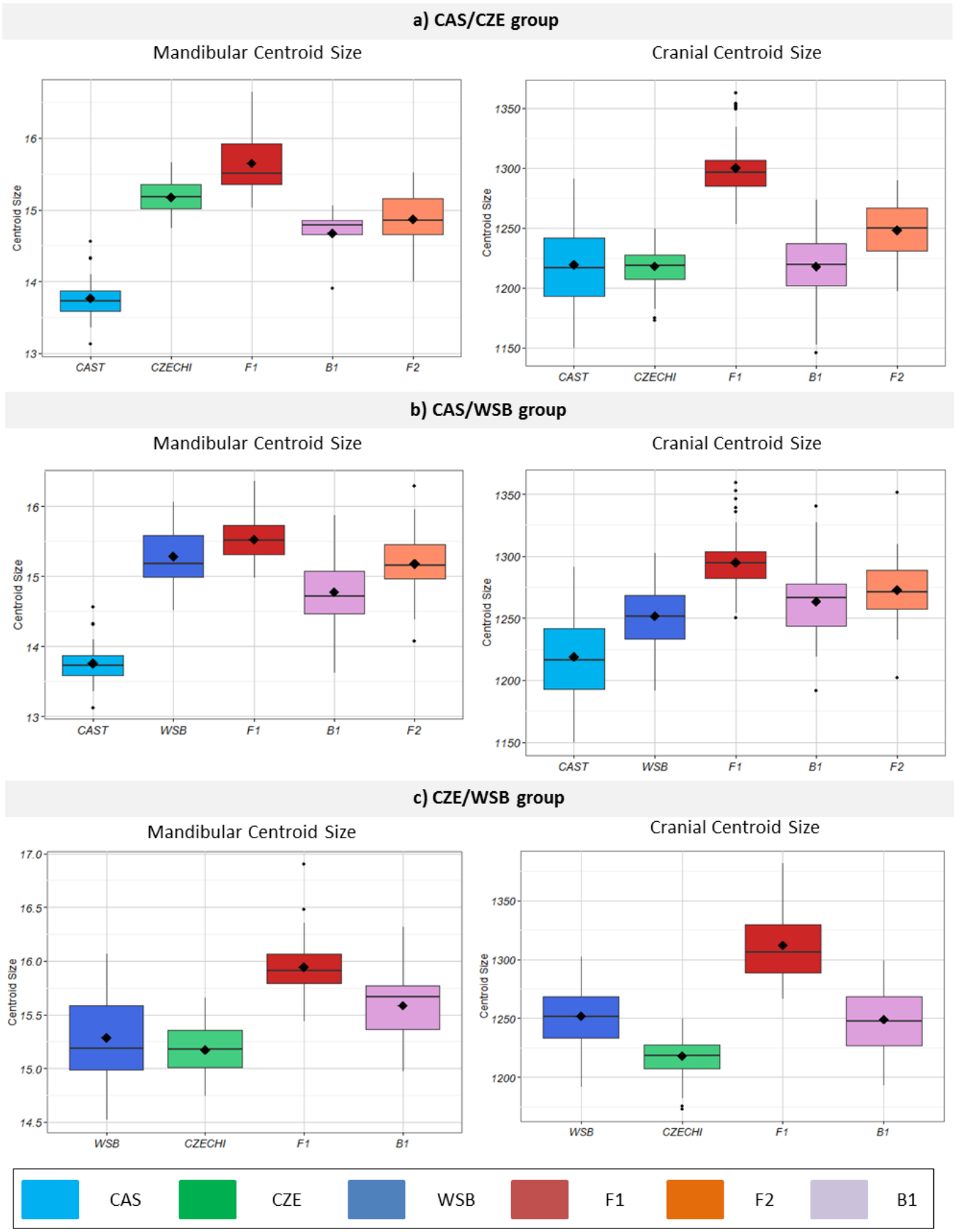


FIGURE 7.1.1. BOX PLOTS OF CENTROID SIZE FOR THE MANDIBLES AND CRANIA OF THE STRAINS WITHIN THE A) CAS/CZE, B) CAS/WSB AND C) CZE/WSB GROUPS.

CLASSIFICATION

Figure 7.1.2 shows the simple classification of individuals within strains to either the parents or F1 hybrids using mandibular and cranial centroid size. It is worth noting that these classifications are simple, and neither group is weighted by variance.

For centroid size in the mandible (left), we see that some individuals are misclassified, but the majority of individuals in the parents and F1 hybrid groups are correctly classified. For instance in the CAS/CZE chart, the parents and F1 strains have individuals that are closer to the average of another group. The patterns for the multi-generation recombinants are more mixed: the F2s in the CAS/CZE group are more like the parents in terms of mandibular size, whereas the F2s in CAS/WSB vary more greatly in terms of whether their size is more parental or F1 hybrid-like. Similarly, we expect size closer to the parents for B1s (particularly resembling the parent with which it was backcrossed). In all three groups, however, the signature is very mixed, with considerable variation in mandibular size (both parental and hybrid-like; CAS/CZE is the exception, but small sample size in this group may affecting these results).

Figure 7.1.2 also shows the classification of individuals within strains to either the parents or F1 hybrids, using the centroid size of cranial form (right). The patterns differ somewhat to those seen in the mandible. Here, the size of individuals in the groups only occasionally resembles the mean of the group to which they belong. Once again, this is likely the result of considerable variation in centroid size within groups, relative to minor variation in the means between groups. In most of the crosses, the majority of individuals are closer to the strain with which they belong. This is not the case for CAS, where most individuals more closely resemble CZE, possibly due to the larger cranial variation of the former strain. The F1s, however, more closely resemble the means of the hybrid-group, no doubt due to hybrids being larger, on average, than either parent. The F1 hybrids which are not classified into the hybrid group, more closely resemble the larger parent in each analysis.

The patterns for the multi-generational recombinants are more mixed: the F2s in the CAS/CZE and CAS/WSB groups vary in terms of parental versus F1 hybrid classification. In the CAS/CZE crosses, the F2 recombinants classified to the parental mean more closely resembled CAS (the smaller parent). In the CAS/WSB cross, the F2 recombinants were more frequently resembled WSB (the larger parent: although not the one with which it was backcrossed, likely due to hybrid heterosis). In the three different crosses, the B1 signature is also mixed. In the CAS/CZE group, B1 is more likely to resemble CAST or the F1. In the CAS/WSB group, it is more likely to resemble WSB or the F1 hybrid. In the CZE/WSB group, it is closer in size to the parent groups. These distributions are a likely result of the extreme size of the F1 hybrids, rather than the affinity to the parent *per se*. For instance, the F1 hybrids

are larger, followed by WSB and then the CAST and CZECHI crania. The size variation in the multigenerational recombinants means that those with closer affinity to CAST or CZECHI are probably just the smaller ones in the range, and those with affinity to F1s the larger.

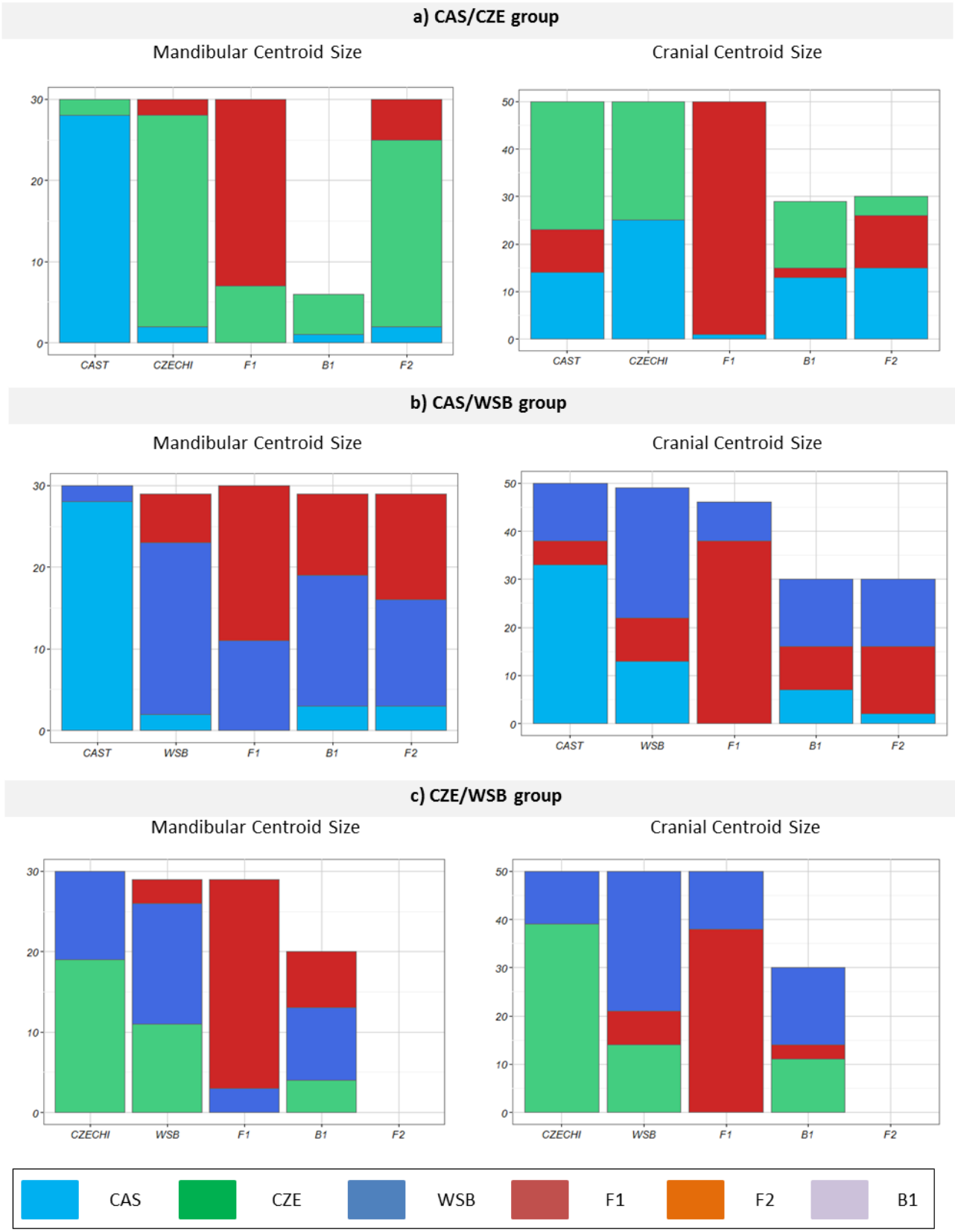


FIGURE 7.1.2. RESULTS OF THE CLASSIFICATION MODEL, AS PERFORMED USING CENTROID SIZE FOR THE MANDIBLE (LEFT) AND CRANIUM (RIGHT). INDIVIDUALS IN EACH STRAIN ARE ASSIGNED AS EITHER PARENT OR F1, BASED ON PROXIMITY TO STRAIN MEAN WITHIN THE A) CAS/CZE, B) CAS/WSB AND C) CZE/WSB GROUPS.

Mixed Model

Figure 7.1.3 shows the plots for the 95% confidence intervals (CIs) for the distribution of means (in red) and variances (in green) in mandible centroid size as the proportion of F1 hybrids (versus a mixed proportion of parents only) in a sample increases. The far left of each graph indicates a sample comprised only of parents (i.e. 0% of the sample contains hybrids), and the far right of the graph a sample with only hybrids. In all three distributions of means plots (left), we see an increase in mandibular centroid size with greater proportion of hybrids (towards the right). Upper confidence intervals of "parents only" means are lower than the lower confidence interval when hybrids make up around 40% of the sample in CAS/CZE and CZE/WSB groups, but not at all in CAS/WSB group. The pattern for variance across the three groups differs somewhat, although the variance increases up until hybrids comprise approximately 50% of the sample (70% for CAS/CZE) and then decreases until hybrids comprise the total sample for all three groups. While in CAS/WSB and CZE/WSB the mixed samples are not necessarily significantly greater at any point than the parents-only, the trend is compelling. Furthermore, in the CAS/CZE group, when 50% of the sample has hybrids, the mean mandibular size is significantly larger in the mixed group than in the parents.

Figure 7.1.4 shows these same figures for cranial centroid size. Here, the patterns are quite consistent across groups for both the mean and variance of the sample cranial size. Similar to the pattern depicted in the mandibular model, means increase with a greater proportion of hybrids making up the sample. For the variances, the pattern is more dome-shaped: increasing towards a point, then decreasing as hybrids begin to dominate the sample. Both trends are comparable to that seen in the mandible. The CIs of the means are also smaller than seen for the mandible. Thus, the cranial centroid sizes of the samples become significantly larger than a mixed parent group when approximately 30% of the sample contains hybrid cranial sizes. In terms of variance, the mixed samples are significantly greater than the combined parent sample at between 20% and 80% hybrids for the CAS/CZE group and CZE/WSB group respectively, but never for the CAS/WSB group.

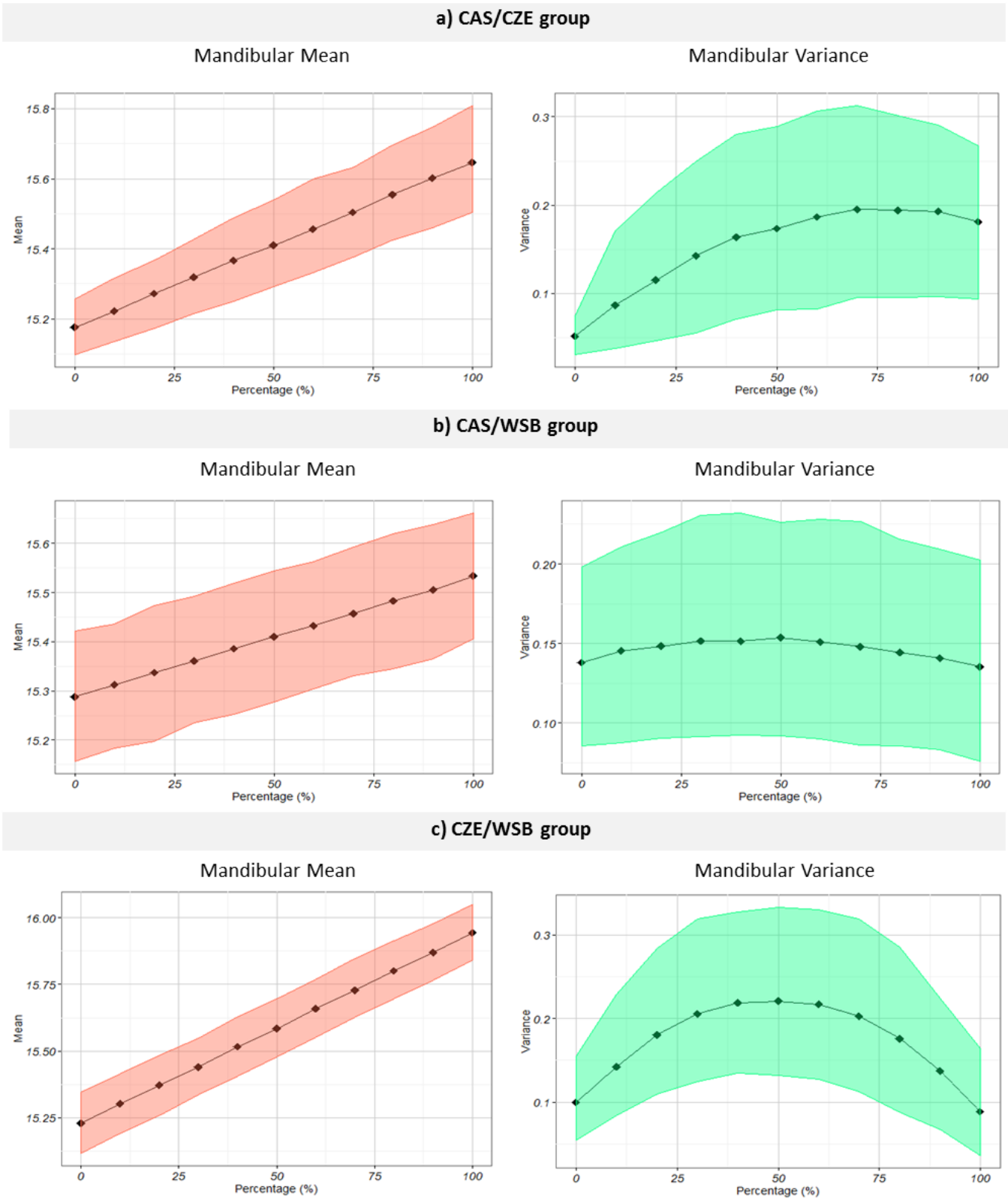


FIGURE 7.1.3. RESULTS OF THE MIXED MODEL FOR MANDIBULAR CENTROID SIZE. THE PLOTS SHOW THE 95% CI OF THE DISTRIBUTION OF MEANS IN RED (LEFT) AND VARIANCES IN GREEN (RIGHT) FOR THE A) CAS/CZE, B) CAS/WSB AND C) CZE/WSB GROUPS, WITH INCREASING PROPORTIONS OF HYBRIDS USED IN THE ANALYSIS FROM LEFT TO RIGHT.

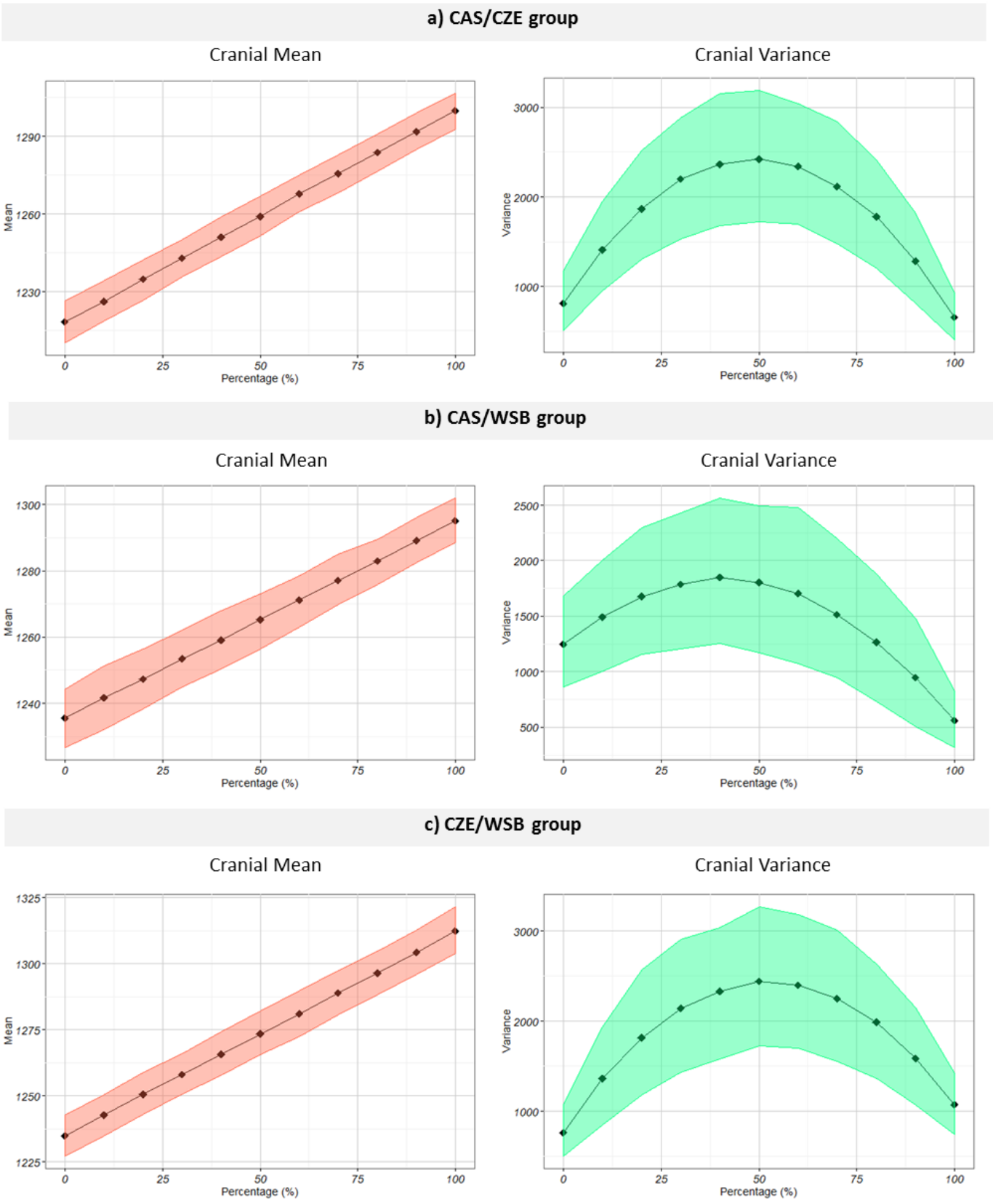


FIGURE 7.1.4. RESULTS OF THE MIXED MODEL FOR CRANIAL CENTROID SIZE. THE PLOTS SHOW THE 95% CI OF THE DISTRIBUTION OF MEANS IN RED (LEFT) AND VARIANCES IN GREEN (RIGHT) FOR THE A) CAS/CZE, B) CAS/WSB AND C) CZE/WSB GROUPS, WITH INCREASING PROPORTIONS OF HYBRIDS USED IN THE ANALYSIS FROM LEFT TO RIGHT.

PART 2: SHAPE ANALYSIS

PRINCIPAL COMPONENTS ANALYSES

Figure 7.2.1 shows the visualisations from three Principal Components Analyses (PCAs) for the three different groups for mandibular shape. The hulls show the extent of the range of individuals per strain in the graphs.

In Figure 7.2.1a, the plot between CAST and CZECHI and their hybrids shows the most separation along PC1 (which accounts for 36.2% of the variance) between individuals from each of the parent groups. The hybrids (F1s, F2s and B1s- backcrossed to CZECHI) are intermediate between the two parent groups, overlapping somewhat with CZECHI but not with CAST (the smaller parent). This may reflect greater allometric shape similarities between the hybrids (which are generally larger) and the larger parent (explored later in this section). The shape change along PC1 shows that morphological differences are mainly in the relative length of the ramus, and the steepness of the coronoid relative to the molar row, with CZECHI having a relatively taller ramus and a steeper coronoid. PC2 (accounting for 17.5% variance) shows the greatest divergence between CZECHI and the F1 and F2 hybrids, with CAST and B1s appearing intermediate. The shape change along PC2 also indicates that steepness of the coronoid is greater in CZECHI than in CAST and the hybrid groups, as well as having a relatively straighter profile along the inferior border of the mandible.

In Figure 7.2.1b, the plot between CAST and WSB shows greatest separation along PC1 (26% variance) between the two parent groups, with hybrids (F1s, F2s and B1s) intermediate (B1s overlapping slightly with CAST- the parent with which the group was backcrossed). The shape change along PC1 shows that WSB has a relatively thicker mandibular body, and steeper coronoid relative to molar row. The mandibular foramen of WSB is more anteriorly positioned, relative to the hybrids, and, especially, relative to CAST. Along PC2 (18.5% variance), there is greater variation among the hybrids (the parents are intermediate), with B1 individuals and F2 individuals at the extremes. The shape change along PC2 shows that these hybrid groups differ primarily along the profile of the inferior border of the mandibular ramus, with the border relatively straighter in F1s compared with B1s.

In Figure 7.2.1c, the plot between CZE and WSB, parent groups once again are the most separated along PC1 (40.6% of the variance). While F1 hybrids are mostly intermediate between parents (one individual overlaps with the WSB cluster –the larger parent), the B1 cluster overlaps with both

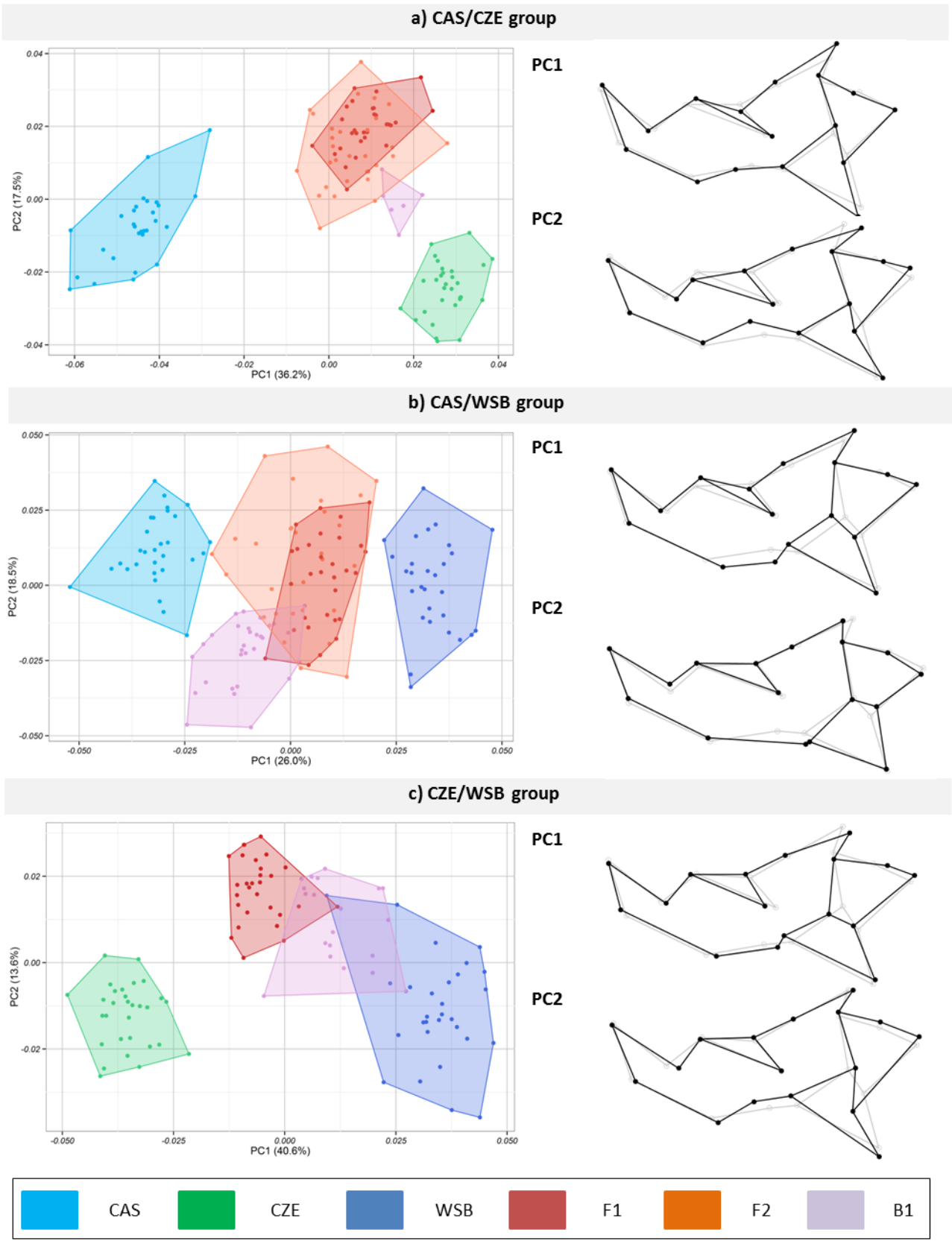


FIGURE 7.2.1. PRINCIPAL COMPONENTS ANALYSES SHOWING DISTRIBUTIONS OF PC1 AND PC2 AMONG STRAINS IN THE A) CAS/CZE, B) CAS/WSB AND C) CZE/WSB GROUPS FOR MANDIBULAR SHAPE, AND THE ASSOCIATED SHAPE CHANGE ASSOCIATED WITH THE FIRST TWO PRINCIPAL COMPONENTS (DEMONSTRATED HERE USING WIREFRAME DIAGRAMS).

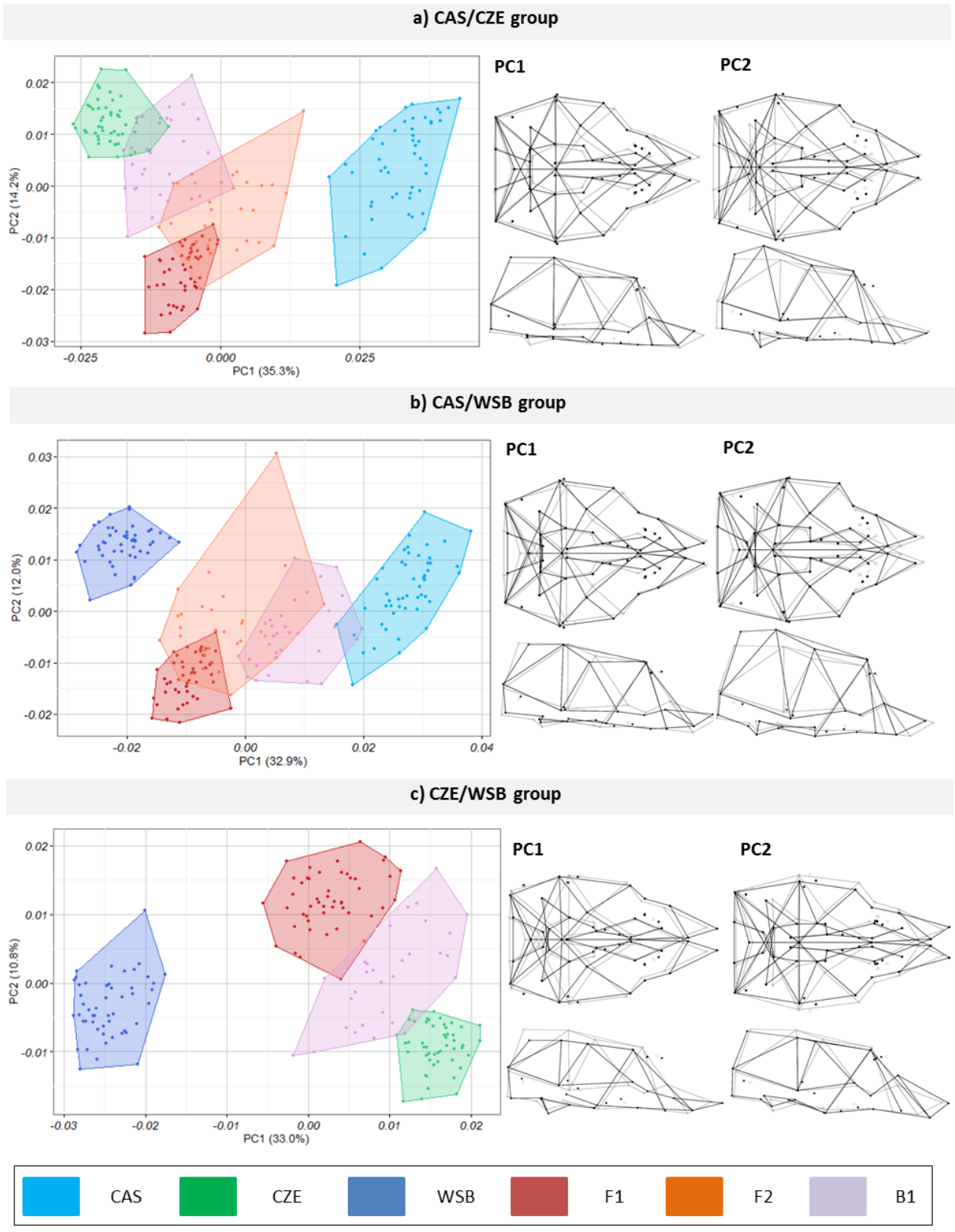


FIGURE 7.2.2. PRINCIPAL COMPONENTS ANALYSES SHOWING DISTRIBUTIONS OF PC1 AND PC2 AMONG STRAINS IN THE A) CAS/CZE, B) CAS/WSB AND C) CZE/WSB GROUPS FOR CRANIAL SHAPE, AND THE ASSOCIATED SHAPE CHANGE ASSOCIATED WITH THE FIRST TWO PRINCIPAL COMPONENTS (DEMONSTRATED HERE USING WIREFRAME DIAGRAMS).

the F1s and WSB (the parent with which they are backcrossed). The shape change along PC1 shows that where the two parents appear to differ greatly is in the angle of the coronoid process, and the angle and relative size of the mandibular condyle. Along PC2 we see a similar trend of separation as we saw in 7.2.1a (the CAS/CZE plot), with the hybrids and parents (particularly WSB) forming the extremes along PC2. The shape change along PC2 shows that the depth of the concavity of the sub-condylar ramus occurs more inferiorly in hybrids than in parents.

It is worth noting the trend seen in all three plots. Firstly, the greater difference, seen in PC1 in all three graphs, exists between parent strains, with shape differences between these groups largely influenced by the angle of the coronoid process relative to the molar row. Furthermore, there tends to be a "hybrid cluster" of F1s, B1s and (where applicable) F2s, which is intermediate to the two parents in PC1. Deviations from this cluster occur where the B1s more closely resemble the parents with which they are backcrossed. In the two plots which show F2 hybrids, the hybrids are more variable (more dispersed) in both PC1 and PC2 than F1s, but there is great overlap between both strains. B1s in all three groups diverge slightly from this "hybrid cluster", often in the direction of the backcrossed parent, especially along PC2 (although also along PC1 in the CZE/WSB cross and the CAS/WSB cross as well). In the CAS/WSB cross, the B1 hybrid not only separates from the "hybrid cluster", but also separates from parent strains along PC2, forming an extreme.

Among the parents, it appears as if CZE has a relatively steep coronoid process and CAST a relatively shallow one. CAST also appears to have a relatively thinner mandibular body, and WSB a comparatively thicker one. In these features hybrids appear to be generally intermediate. Relative concavity of the posterior border of the mandibular ramus differs between WSB and hybrids, and CZECHI and hybrids, along PC2 (potentially musculature due to larger size).

Figure 7.2.2 shows the PCAs of each of the three intraspecific groups for the Procrustes-aligned cranial coordinates. Like in the mandible, the patterns in each graph are very similar to each other. In each graph, the largest portion of form variance appears to be between the parent strains, along PC1. The hybrids, in each case, are intermediate. Along PC2, all the hybrids, or some subset of them, tend to be extreme, relative to the parents.

In Figure 7.2.2a, the largest differences along PC1 (which accounts for 35.3% of the variance) are between the parents, with F1 hybrids largely intermediate, but also overlapping with CZE (the larger strain). The differences between the parents reflect differences in the width of the mid-cranial region, and in relative snout length. Differences along PC2 (which accounts for 14.2% of the variance, and where the F1 hybrids form one extreme) reflect differences in the basicranial region and in the relative

projection of the face and height of the cranium. The F2s appear to have large variance along both PC1 and PC2, intermediate between both parents along PC1 and intermediate between the F1 hybrids (which form one extreme) and CZE (which forms the other along PC2). The backcrossed strain, to CZE, appears to overlap more with the F1s and CZE along PC1 and the parents along PC2.

In Figure 7.2.2b, we see a similar pattern, with parents forming the extreme along PC1 (32.9%). The CASxWSB F1 hybrids are largely intermediate, overlapping with WSB on PC1. Along PC1, the separation between the parents appears to reflect relative elongation of the snout and length and width of the midcranial region, with some relative pinching on the basicranium. F1 individuals along PC2 (12%) form one extreme, while parental individuals (and one F2 individual) form the other (although all groups are widely spread over this PC. Along PC2, this appears to reflect relative projection of the face and snout (hybrids longer) and relative length of the basicranium and height of the neurocranium. The F2s are highly variable along both PC1 and PC2. On PC1, they are intermediate between the parents, with larger expanse than the F1s. On PC2, they overlap with both the parents and F1s, and, for one individual, at the extreme along PC2. The CAST backcrosses also appear more variable than the F1s, spanning the space between the F1s and overlapping with CAST along PC1, and overlapping with both parents (more so with CAST) and F1s along PC2. CASxWSB F2 12 is an outlier along PC2 (an individual that, otherwise, looks normal in CT scans, and whose landmarks do not otherwise stand out).

In Figure 7.2.2c, the F1s are intermediate between the parents, but overlap slightly with CZE along PC1 (33%). Along PC1, the parents differ in relative width/length ratio of the cranium, with some differences in the basicranium and cranial height. Along PC2 (10.8%), the F1s form one extreme, and CZECHI individuals the other (although WSB and CZECHI largely overlap). Some WSB and F1 individuals and F1s also overlap along PC2.In both PC1 and PC2, the B1 sample is intermediate and overlaps with the F1s and the backcrossed parent (CZECHI). Along PC2, the hybrids appear to show relatively longer snouts and thinner faces.

There does appear to be a trend in all three cranial plots, similar to those described for the mandible. The greater difference, seen in PC1 in all three graphs, exists between parent strains, with shape differences mainly in snout length and width and cranial height. Similar to the trends in the mandible, a “hybrid cluster” of F1s, B1s and (where applicable) F2s exists intermediate to the two parents in PC1. Hybrid individuals form one extreme in PC2 in all three PCAs, and B1s and F2s overlap with both parents and F1s in PC2.

ALLOMETRY

Figure 7.2.3 shows the regression plots (mandibular and centroid size against regression score) for each of the three groups, as calculated in MorphoJ. In each analysis, size is a significant predictor of shape ($p<0.0001$). However, the amount of shape it predicts differs for each analysis: in the CAS/CZE analysis, size accounts for 22.9% of the predicted mandibular shape, but it is 10.5% for CAS/WSB and 7% for WSB/CZE. This is also possibly a reflection of the size differences between the different parents, with CAST being significantly smaller than either CZECHI or WSB (see Figure 7.1.1). While change in size has an effect on size throughout the mandible, it appears most profound along the inferior and posterior borders of the mandible, particularly around the coronoid process and mandibular condyles (see wireframes in Figure 7.2.3: magnified 0.3). Among the crosses that contain CAST, the relative angle of the molar alveolar also differs among groups.

Figure 7.2.3 also shows the cranial regression of each of the hybrid group comparisons. In each comparison, size accounts for 12.3%, 13.5% and 8.4% of the form analysed in the CAS/CZE, CAS/WSB and CZE/WSB groups, respectively. In each analysis, the shape change along centroid size seems to reflect lengthening and thinning of the face and cranium.

When comparing mandibular shape and size with strain as a cofactor, we see an interesting pattern in all three groups (Table 7.2.1). Homogeneity of slopes indicates that the allometries of strains are not parallel in any of the group comparisons ($p<0.0001$). Similarly, as was seen in the direct allometric comparisons, size accounts for 23% of the shape variation in CAS/CZE, 15.5% in CAS/WSB, and 7% in CZE/WSB. The strain however, contributes a larger proportion of the differences in all three comparisons: 30.5%, 24% and 45% in the order as above (no doubt the larger proportion in the CZE/WSB comparison is due to the parents being of similar size, whereas CAST is a clear outlier in the other analyses). Within group size only accounts for 2-3% of the shape variation.

When comparing cranial shape and size with strain as a cofactor, we again see an interesting pattern in all three groups (Table 7.2.2). Homogeneity of slopes indicates that the allometries of strains are not parallel in any of the group comparisons ($p< 0.0001$). Similarly, as was seen in the direct allometric comparisons, size accounts for 13% of the shape variation in CAS/CZE, 14% in CAS/WSB, and 8% in CZE/WSB. The strain however, contributes a larger proportion of the differences in all three comparisons: 39%, 31% and 37% in the order as above. Size within group only accounts for 1.8-2.2% of the shape variation.

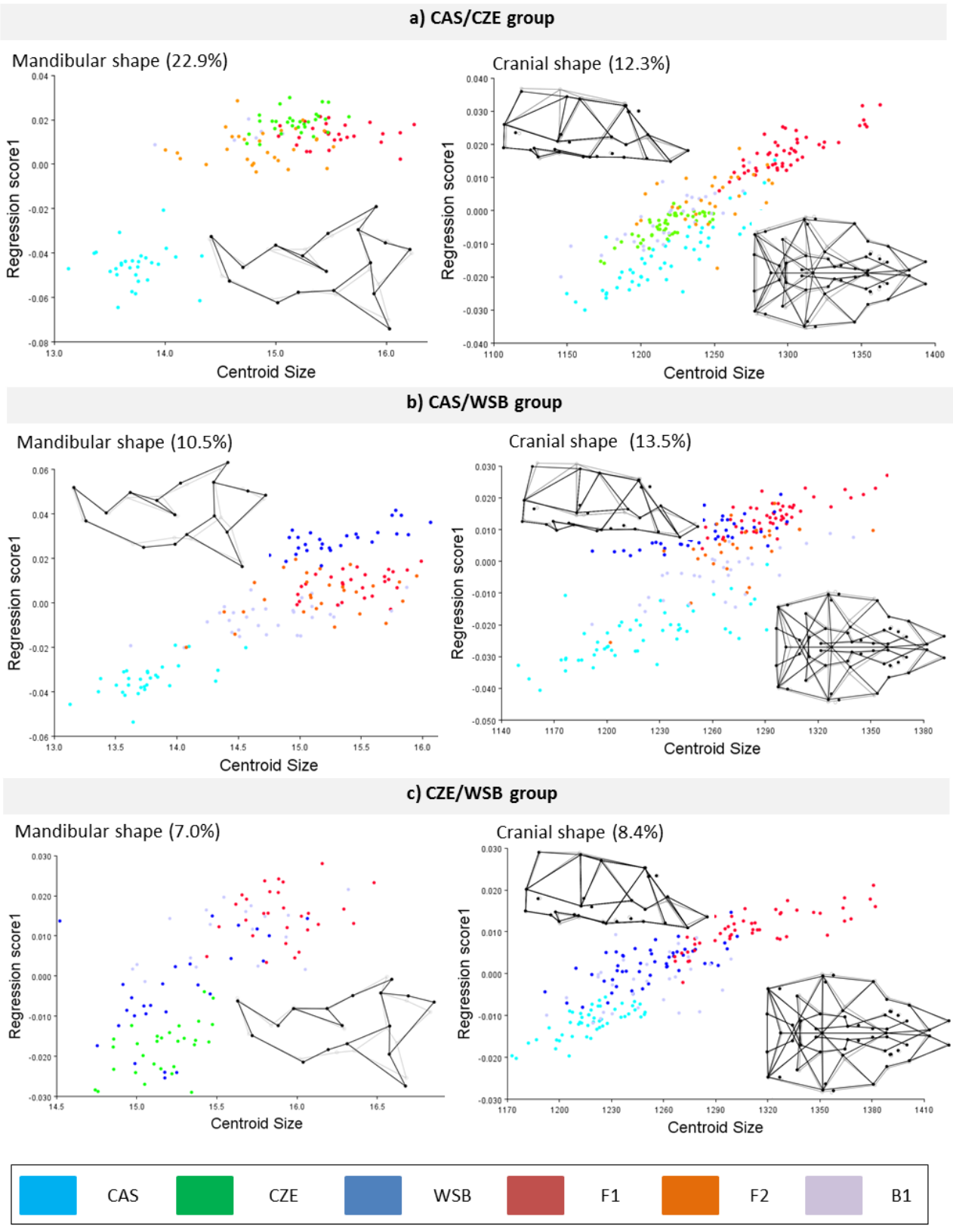


FIGURE 7.2.3. REGRESSION CHARTS SHOWING THE SIZE-ASSOCIATED SHAPE CHANGE IN THE MANDIBLE (LEFT) AND CRANIUM (RIGHT) FOR THE A) CAS/CZE, B) CAS/WSB AND C) CZE/WSB GROUPS, AND THE ASSOCIATED SHAPE CHANGE (DEMONSTRATED HERE USING WIREFRAME DIAGRAMS).

TABLE 7.2.1 ANOVA TABLES FOR EACH REGRESSION COMPARISON OF MANDIBULAR SHAPE VS SIZE AND STRAIN.

CAS/CZE comparison							
	Df	**SS**	**MS**	**Rsq**	**F**	**Z**	**Pr(>F)**
log(size)	1	0.060905	0.060905	0.23357	62.4935	24.2693	1.00E-04
Strain	4	0.079575	0.019894	0.305168	20.4126	11.9525	1.00E-04
log(size):Strain	4	0.008201	0.00205	0.031449	2.1036	2.0823	1.00E-04
Residuals	115	0.112077	0.000975				
Total	124	0.260759					
CAS/WSB comparison							
	Df	**SS**	**MS**	**Rsq**	**F**	**Z**	**Pr(>F)**
log(size)	1	0.044486	0.044486	0.155131	37.1152	20.3705	1.00E-04
Strain	4	0.069671	0.017418	0.242958	14.5319	10.2825	1.00E-04
log(size):Strain	4	0.0072	0.0018	0.025109	1.5018	1.5041	8.50E-03
Residuals	138	0.165405	0.001199				
Total	147	0.286762					
CZE/WSB comparison							
	Df	**SS**	**MS**	**Rsq**	**F**	**Z**	**Pr(>F)**
log(size)	1	0.013849	0.013849	0.06996	15.649	6.3018	1.00E-04
Strain	3	0.090644	0.030215	0.45789	34.14	16.2445	1.00E-04
log(size):Strain	3	0.004968	0.001656	0.02509	1.871	1.8616	0.0014
Residuals	100	0.088502	0.000885				
Total	107	0.197963					

TABLE 7.2.2 ANOVA TABLES FOR EACH REGRESSION COMPARISON OF CRANIAL SHAPE VS SIZE AND STRAIN

CAS/CZE comparison							
	Df	**SS**	**MS**	**Rsq**	**F**	**Z**	**Pr(>F)**
log(size)	1	0.017267	0.017267	0.12892	53.5393	22.9824	1.00E-04
Strain	4	0.05011	0.012528	0.37413	38.8439	20.9333	1.00E-04
log(size):Strain	4	0.002383	0.000596	0.01779	1.8468	1.8467	1.00E-04
Residuals	199	0.064179	0.000323				
Total	208	0.133939					
CAS/WSB comparison							
	Df	**SS**	**MS**	**Rsq**	**F**	**Z**	**Pr(>F)**
log(size)	1	0.017212	0.017213	0.14043	54.1001	25.7999	1.00E-04
Strain	4	0.038962	0.00974	0.31787	30.615	18.5274	1.00E-04
log(size):Strain	4	0.002764	0.000691	0.02255	2.1722	2.1589	1.00E-04
Residuals	200	0.063632	0.000318				
Total	209	0.122571					
CZE/WSB comparison							
	Df	**SS**	**MS**	**Rsq**	**F**	**Z**	**Pr(>F)**
log(size)	1	0.012312	0.012312	0.08415	27.1108	13.3756	1.00E-04
Strain	3	0.053653	0.017884	0.36674	39.3825	22.2517	1.00E-04
log(size):Strain	3	0.002226	0.000742	0.01522	1.6339	1.6376	1.00E-04
Residuals	172	0.078109	0.000454				
Total	179	0.1463					

PCA ON REGRESSION

Figure 7.2.4 shows the Principal Components Analyses for the mandibular regression residuals of each group (PC1 vs PC2) in order to better visualize shape change by minimizing allometric size effects.

In the CAS/CZE group (Figure 7.2.4a), the primary separation in PC1 (28.5% of the variance) appears to be between the parents, with the F1 hybrids appearing parental (similar position to CAS) and the F2 and B1s moving into more intermediate positions. Shape differences along PC1 seem to reflect the angle of the coronoid process and the relative height of the mandibular body, similar to the previous PCAs. For PC2 (14.3%), the strains overlap, with CAST individuals and F2/B1 individuals forming the extremes, and F1 hybrids and CZECHI appearing largely intermediate. The shape change seen in PC2 appears to reflect the height and length of the coronoid process and the inferior border of the mandibular body.

In the CAS/WSB group (Figure 7.2.4b), the separation along PC1 (22.3%) appears to be mostly of individuals within groups than a particular clustering of groups (although it should be noted that B1s form an extreme). The shape change best associated with this is along the inferior border of the mandibular body. It is worth noting that individuals in B1 and F2 form the extremes along PC1, with parental individuals intermediate. Separation along PC2 (15.9%) appears to be largely between WSB (a parent) and the F2s, with F1s and CAST (the other parent) intermediate. Here, the shape change largely reflects the height of the mandibular body.

In the CZE/WSB group (Figure 7.2.4c), separation along PC1 (43.5%) is most predominant between the parent groups, with F1 hybrids intermediate to the two groups, and B1s overlapping with F1s and the WSB parent (with which it is backcrossed). The shape change along PC1 largely reflects the angle of the coronoid process and the inferior border of the mandibular body. PC2 in the CZE/WSB group appears to reflect differences between individuals within strains, with individuals in WSB forming both extremes. It is worth noting that, perhaps congruent with the pattern seen in the other two PCAs, hybrids (F1s and B1s) overlap more greatly with one extreme, and CZECHI (the parent) intermediate. The shape change along PC2 here largely reflects differences in the posterior border of the mandible and the position of the mental foramen.

There is not much similarity among the three mandibular PCA scatterplots. CAS/CZE and CZE/WSB seem to have similar patterns to that seen in the PCAS in Figure 7.2.1. The CASxWSB group, however, appears to have far more overlap between groups.

Figure 7.2.5 shows the PCAs of each of the groups on the cranial regression residuals, again in order to better visualize shape change, taking into consideration the allometric size effects.

In Figure 7.2.5a, PC1 of the CAS/CZE group (accounting for 39.3% of the variance), indicates differences between the two parent strains. Along PC, there appears to be changes in facial width, and projection, and cranial height. There appears to be much greater overlap between strains along PC2 (which accounts for 6.8% of the variance). However, recombinants (particularly F2), appear to be more clustered on the one side of PC2, although overlapping with many parent individuals. Shape change along PC2 is in mid-cranial height, snout projection and around the occipital area.

In Figure 7.2.5b, of the CAS/WSB group, parent strains are separated along PC1 (accounting for 30.6% of the variance), and hybrids are largely intermediate (a small overlap with CAS). Along this PC, posterior cranial height and anterior temporal projection appears to change. Along PC2 (8.8% of variance), there is much overlap among all groups, however, similar to the pattern seen in the CAS/CZE group, the hybrids are largely clustered around one extreme. Along PC2, cranial height, temporal flare and snout projection appears most affected.

In Figure 7.2.5c, the PCA for the CZE/WSB group, PC1 (35.7% of variance) largely separates WSB from CZE and the hybrids. The F1 hybrids overlap with CZE, but are spread into the intermediate space between CZE and WSB. The B1 hybrids are more variable along PC1, overlapping with both F1s and CZE. Along this PC, cranial height and snout projection appear most affected. Along PC2 (7.1% of variance), there is large overlap between all of the strains, explaining differences in facial and cranial width.

The patterns are largely similar to that seen in the earlier cranial PCA (Figure 7.2.2); the greatest separation along PC1 in all three comparisons appears to be between parent strains, with hybrids intermediate to the two groups. Additionally, the hybrids appear to overlap with CAST in the CAS/WSB comparison (7.2.5b) and with CZECHI in the CZE/WSB comparison (7.2.5c). These are the smaller strains in these comparisons, and may reflect that, without size, the hybrid more closely resembles the smaller parent in shape, or that WSB as a strain does not have highly heritable effects on shape (which is also likely since the pattern is not reflected in the CAS/CZE group). In general, without size, the strains and hybrids appear to overlap far more closely, particularly along PC2, where the F1 hybrids are not a clearly segregated extreme, and the variation along this axis primarily reflects differences among individuals within strains.

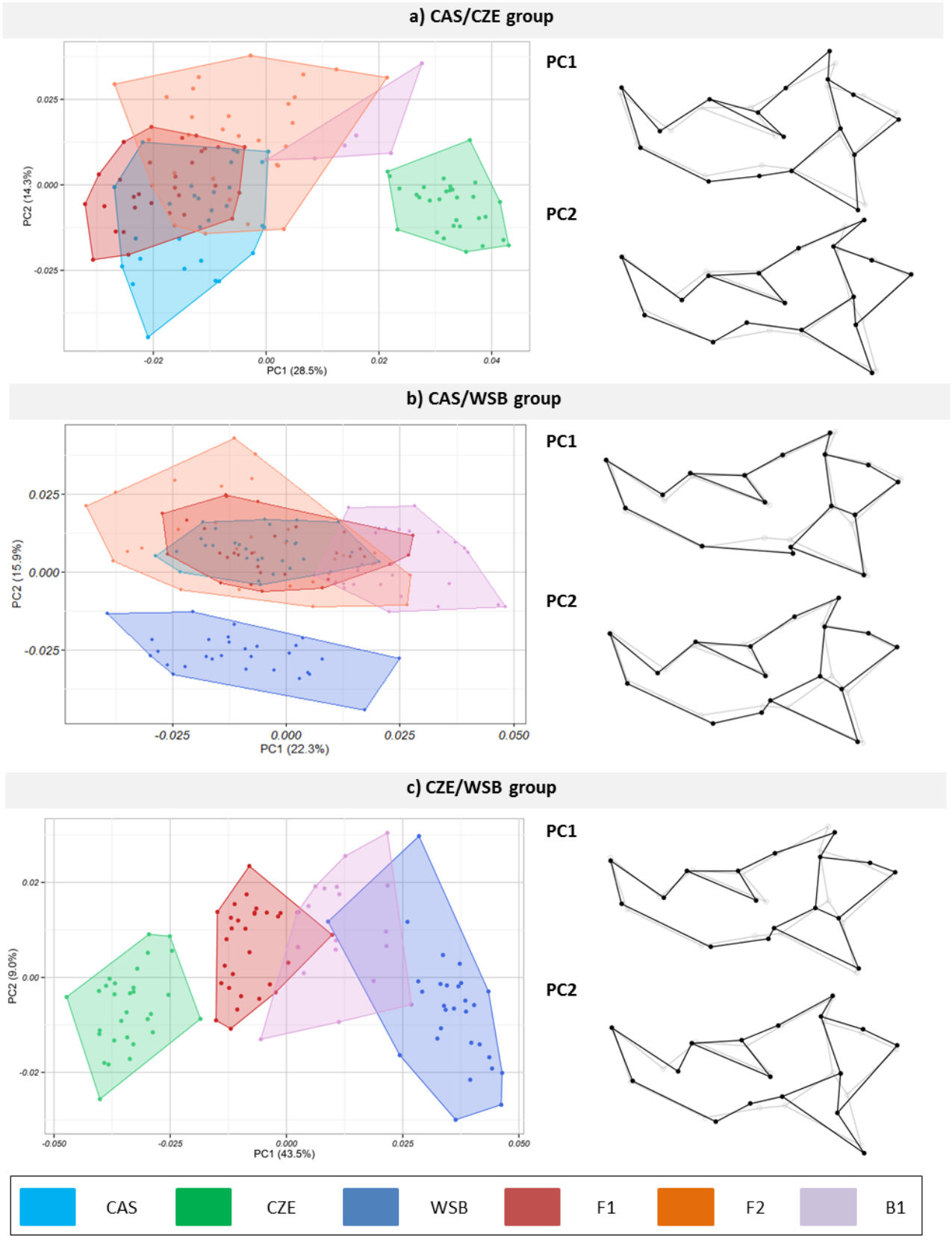


FIGURE 7.2.4. PRINCIPAL COMPONENTS ANALYSES ON THE REGRESSION SCORES OF MANDIBULAR SHAPE, SHOWING DISTRIBUTIONS OF PC1 AND PC2 AMONG STRAINS IN THE A) CAS/CZE, B) CAS/WSB AND C) CZE/WSB GROUPS, AND THE ASSOCIATED SHAPE CHANGE (DEMONSTRATED HERE USING WIREFRAME DIAGRAMS).

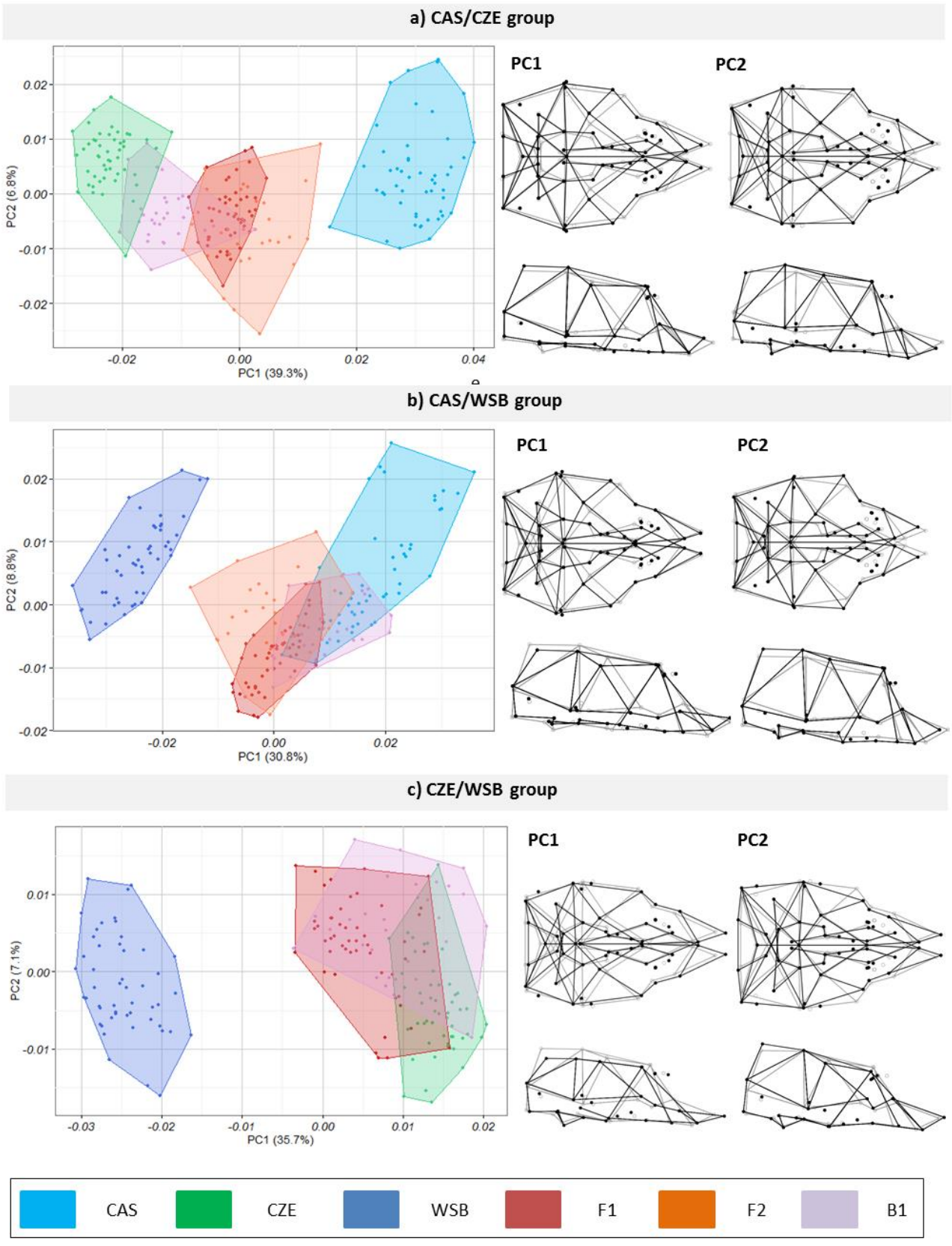


FIGURE 7.2.5. PRINCIPAL COMPONENTS ANALYSES ON THE REGRESSION SCORES OF CRANIAL SHAPE, SHOWING DISTRIBUTIONS OF PC1 AND PC2 AMONG STRAINS IN THE A) CAS/CZE, B) CAS/WSB AND C) CZE/WSB GROUPS, AND THE ASSOCIATED SHAPE CHANGE (DEMONSTRATED HERE USING WIREFRAME DIAGRAMS).

PART 3: DISPARITY AMONG GROUPS

CANONICAL VARIATES ANALYSES

Canonical variates analyses (CVAs) of the mandibles are displayed in Figure 7.3.1. The strains are distributed similarly in all three plots. CV1, which accounts for 75.5% of the variance in the CAS/CZE groups, 70.4% in the CAS/WSB groups and 76.3% in the CZE/WSB groups, clearly showing that the primary separation is between the parent strains, with hybrids (F1s, F2s and B1s) intermediate, and B1s "drifting" more closely to the parent with which they are backcrossed. The shape change, which this best represents, is in the coronoid height or projection in the three different groups.

By contrast, along CV2, which accounts for 19.2% of the variance in the CAS/CZE groups, 16.5% in the CAS/WSB groups and 13% in the CZE/WSB groups, the majority of separation is between the hybrids and parents. The shape change along CV2 in each cross shows differences in the angle of the coronoid process, height of the body of the mandible and posterior condylar projection.

Table 7.3.1 shows the Mahalanobis and Procrustes distances of shape between each of the groups in the three analyses. In all three analyses, the greatest distance in shape exists between the parent groups. The second greatest distance between groups often is between the B1 and the parent with which the group has not been backcrossed. Among the hybrids, the Mahalanobis and Procrustes distances between F1, F2 and B1 is far smaller, with the distances between the CAS/CZE B1 and F2 groups, and between the CAS/WSB F1 and F2 groups not significant on the Bonferroni-corrected p-value ($p = 0.0027$ and 0.0037, respectively, with the corrected $p=0.0005$).

Similarly, the CVA analyses for the crania (Figure 7.3.2) allow us to visualise the specific differences among strains within each group. The pattern is similar to that seen in the PCAs and fairly consistent among all three groups: the largest separation occurs between the parents along CV1 (which accounts for 77.3%, 70.5% and 85.8% of the variance between the CAS/CZE, CAS/WSB and CZE/WSB groups). CV1 accounts for a larger proportion of the variance due to only having three groups with which to differentiate. The differences among parents in each of the three groups largely reflect subtle differences in relative width of the mid-cranial region, lateral temporal projection (anteriorly) and relative length of the snout.

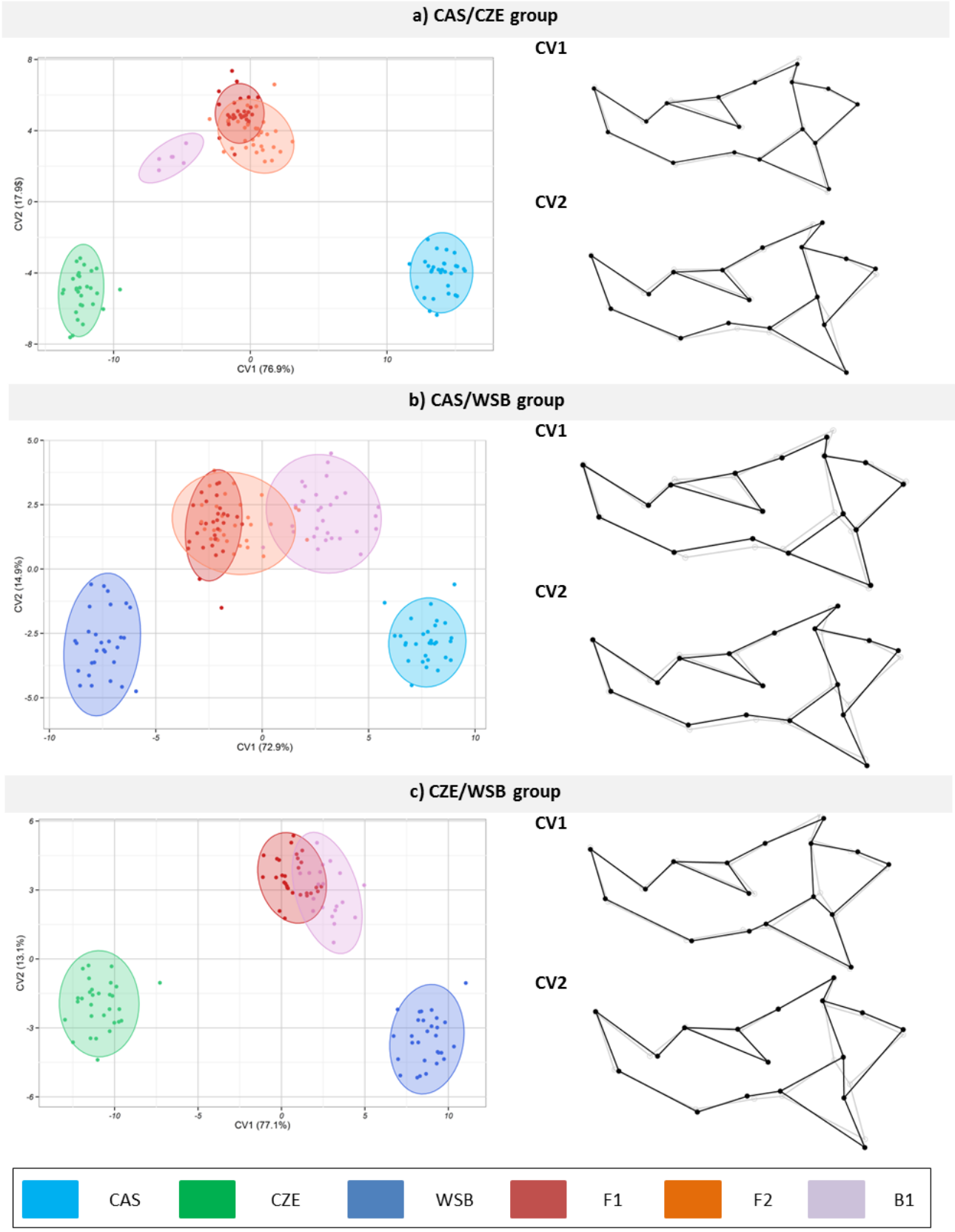


FIGURE 7.3.1. CANONICAL VARIATES ANALYSES FOR MANDIBULAR SHAPE, SHOWING DISTRIBUTIONS OF CV1 AND CV2 AMONG STRAINS IN THE A) CAS/CZE, B) CAS/WSB AND C) CZE/WSB GROUPS, AND THE ASSOCIATED SHAPE CHANGE (DEMONSTRATED HERE USING WIREFRAME DIAGRAMS).

TABLE 7.3.1. MATRICES SHOWING MAHALANOBIS DISTANCE BETWEEN GROUPS BELOW HORIZONTAL AND PROCRUSTES DISTANCES ABOVE HORIZONTAL. P-VALUES FROM PERMUTATION TESTS (10000 PERMUTATION ROUNDS FOR BOTH MAHALANOBIS AND PROCRUSTES DISTANCES; IF P< 0.05 UNDERLINED, IF P<0.001 THEN ITALICS, IF P<0.0001 THEN BOLD).

Distances between CAS/CZE groups					
	CAST	CZECHI	F1	F2	B1
CAST	–	**0.0759**	**0.0629**	**0.0593**	**0.0679**
CZECHI	**25.7034**	–	**0.0496**	**0.0466**	**0.0344**
F1	**17.2774**	**15.3592**	–	**0.0225**	**0.0325**
F2	**16.7085**	**15.2865**	**6.0531**	–	*0.0292*
B1	**21.8406**	**10.9847**	**9.4862**	**8.5087**	–
Distances between CAS/WSB groups					
	CAST	WSB	F1	F2	B1
CAST	–	**0.0659**	**0.0448**	**0.0423**	**0.0455**
WSB	**14.7806**	–	**0.0341**	**0.0407**	**0.0533**
F1	**10.7454**	**7.7198**	–	*0.0184*	**0.0374**
F2	**9.9479**	**8.6956**	**4.6596**	–	**0.0384**
B1	**7.5745**	**11.394**	**6.8088**	**5.8725**	–
Distances between CZE/WSB groups					
	CZECHI	WSB	F1	B1	
CZECHI	–	**0.0706**	**0.0432**	**0.0562**	
WSB	**18.8654**	–	**0.0508**	**0.0372**	
F1	**11.9271**	**11.2447**	–	**0.0284**	
B1	**15.0523**	**9.8573**	**8.6799**	–	

The shape change along CV2 is important to note, considering it accounts for the differences between parents and F1 hybrids. In all three CVAs, the relative length of the snout, cranial height, and projection and width of the face seems to reflect the shape change along CV2. In the CAS/CZE cross, CV2 also reflects relative “pinching” in the mid-basicranial region of the skull.

Table 7.3.2 shows the Mahalanobis and Procrustes distances between the strains within each of the groups. All group comparisons are significant at p<0.0001 for cranial form. In each case, the greatest form difference in each group appears between the parent strains, as reflected in the CVAs. This is followed by the backcross to the parent with which it is not backcrossed in the CAS/CZE and CAS/WSB comparison. The third largest difference is often between the hybrid and one or both of the parents, followed closely by the F2 and the parents. The smallest differences are between the F2 and F1, the F2s and B1s, and the B1s and the parent with which it is backcrossed. However, these distances are still significantly different.

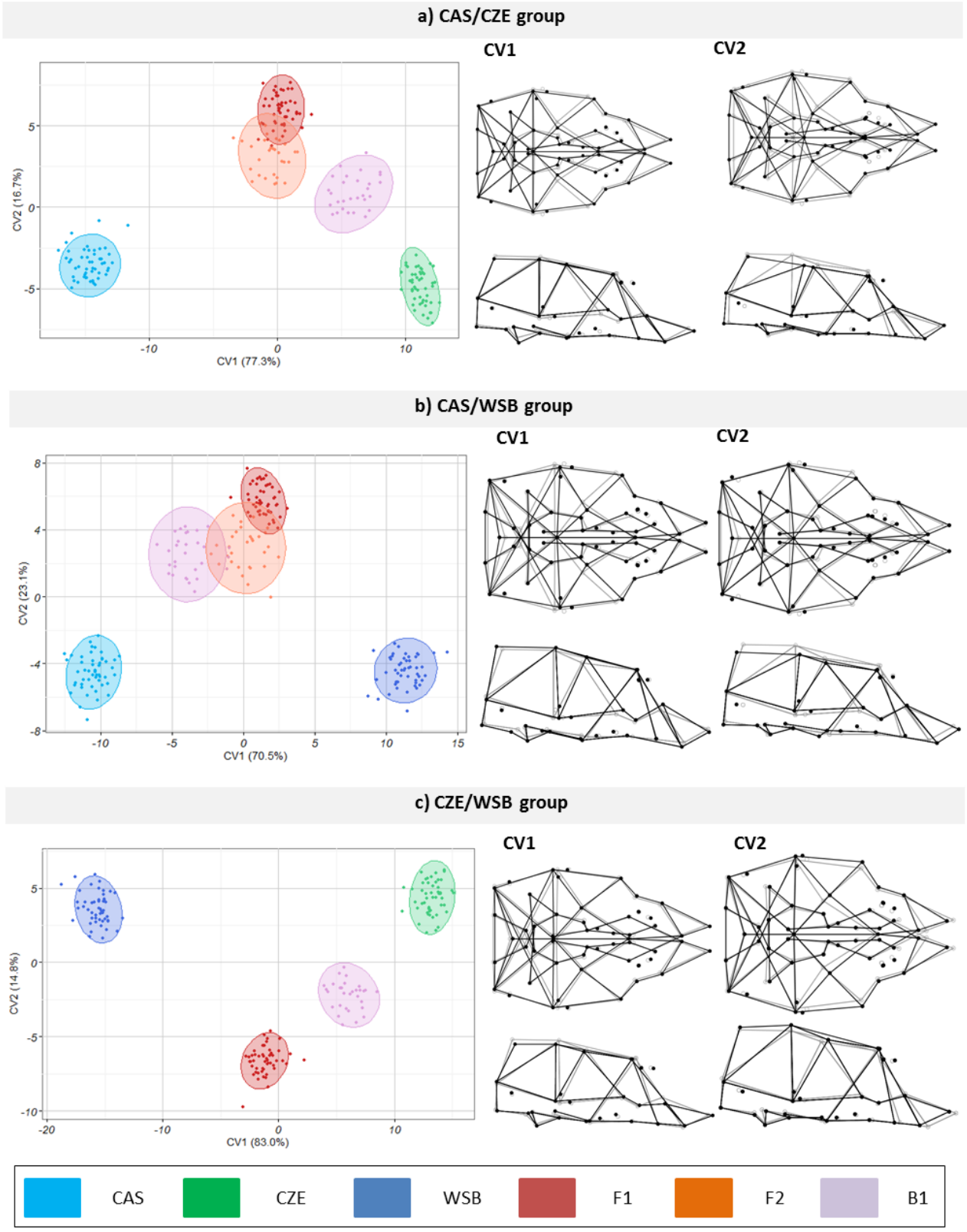


FIGURE 7.3.2. CANONICAL VARIATES ANALYSES FOR CRANIAL SHAPE, SHOWING DISTRIBUTIONS OF CV1 AND CV2 AMONG STRAINS IN THE A) CAS/CZE, B) CAS/WSB AND C) CZE/WSB GROUPS, AND THE ASSOCIATED SHAPE CHANGE (DEMONSTRATED HERE USING WIREFRAME DIAGRAMS).

TABLE 7.3.2. MATRICES SHOWING MAHALANOBIS DISTANCE BETWEEN GROUPS BELOW HORIZONTAL AND PROCRUSTES DISTANCES ABOVE HORIZONTAL. P-VALUES FROM PERMUTATION TESTS (10000 PERMUTATION ROUNDS FOR BOTH MAHALANOBIS AND PROCRUSTES DISTANCES; IF P< 0.05 UNDERLINED, IF P<0.001 THEN ITALICS, IF P<0.0001 THEN BOLD).

Distances between CAS/CZE groups					
	CAST	CZECHI	F1	F2	B1
CAST	–	**0.053**	**0.044**	**0.035**	**0.044**
CZECHI	**25.895**	–	**0.033**	**0.030**	**0.017**
F1	**17.776**	**15.458**	–	**0.018**	**0.025**
F2	**16.428**	**15.084**	**7.382**	–	**0.020**
B1	**21.407**	**9.128**	**9.195**	**9.236**	–
Distances between CAS/WSB groups					
	CAST	WSB	F1	F2	B1
CAST	–	**0.049**	**0.041**	**0.034**	**0.024**
WSB	**21.886**	–	**0.029**	**0.026**	**0.035**
F1	**15.821**	**14.391**	–	**0.017**	**0.022**
F2	**13.749**	**13.955**	**6.609**	–	**0.017**
B1	**10.653**	**16.974**	**8.032**	**6.685**	–
Distances between CZE/WSB groups					
	CZECHI	WSB	F1	B1	
CZECHI	–	**0.041**	**0.025**	**0.016**	
WSB	**28.88**	–	**0.032**	**0.036**	
F1	**18.071**	**17.831**	–	**0.016**	
B1	**10.866**	**22.757**	**9.87**	–	

Within this scenario, the Mahalanobis and Procrustes distances between the parents is therefore the greatest (52.1 and 0.067, respectively), and between the F1 hybrid and WSB is the smallest (29.3 and 0.041). All these distances between groups are significant at p<0.0001. Shape change along PC1 (between parents, with hybrids as intermediate) seem to be throughout the cranium, particularly in snout length and temporal landmark positions. Shape change along CV2 is similar to that seen in the PCA as well: hybrids displaying a thinner face and anterior temporal region, with a longer relative snout and shorter relative cranium.

PROCUSTES VARIANCES

Figure 7.3.3 shows the bar graphs for the cranial and mandibular Procrustes variances of strains within groups. In each graph, the largest Procrustes variances are within the F2 groups (where applicable, i.e. CAS/CZE and CAS/WSB), followed by B1s. Pairwise absolute differences between Procrustes variances, and significance between groups, are shown in matrices in Table 7.3.3. In the two groups which include F2s (CAS/CZE and CAS/WSB), the F2s show significantly larger mandibular Procrustes variances ($p<0.01$) compared to parents and F1 hybrids (but not compared to B1s). This shows that, while F1 hybrids show similar Procrustes variances than the parents (and are therefore themselves not necessarily more morphologically variable as a single group), recombinants are more variable; F2s are significantly so.

TABLE 7.3.3. MATRICES (MANDIBULAR LEFT AND CRANIAL RIGHT) SHOWING THE PAIRWISE ABSOLUTE DIFFERENCES BETWEEN THE PROCRUSTES VARIANCES OF THE STRAINS (IF P< 0.05 UNDERLINED, IF P<0.001 THEN ITALICS, IF P<0.0001 THEN BOLD).

	Mandibular						Cranial				
CAS/CZE group											
	CAST	**CZECHI**	**F1**	**F2**	**B1**		**CAST**	**CZECHI**	**F1**	**F2**	**B1**
CAST	–					**CAST**	–				
CZECHI	0.0001	–				**CZECHI**	**0.0056**	–			
F1	0.0001	0.000	–			**F1**	**0.0053**	0.0003	–		
F2	0.0009	0.0009	0.001	–		**F2**	*0.0039*	**0.01**	**0.0092**	–	
B1	0.0001	0.0002	0.0002	0.0008	–	**B1**	0.0005	**0.0061**	**0.0057**	*0.0034*	–
CAS/WSB group											
	CAST	**WSB**	**F1**	**F2**	**B1**		**CAST**	**WSB**	**F1**	**F2**	**B1**
CAST	–					**CAST**	–				
WSB	0.0001	–				**WSB**	**0.0048**	–			
F1	0.0001	0.000	–			**F1**	**0.0063**	0.0015	–		
F2	0.0009	0.0007	0.0008	–		**F2**	0.0035	**0.0082**	**0.0097**	–	
B1	0.0006	0.0004	0.0005	0.0003	–	**B1**	0.0005	**0.0042**	**0.0057**	*0.004*	–
CZE/WSB group											
	CZECHI	**WSB**	**F1**	**B1**			**CZECHI**	**WSB**	**F1**	**B1**	
CZECHI	–					**CZECHI**	–				
WSB	0.0002	–				**WSB**	0.0008	–			
F1	0.0001	0.0002	–			**F1**	0.0005	0.0004	–		
B1	0.0003	0.0001	0.0003	–		**B1**	**0.0061**	**0.0053**	**0.0057**	–	

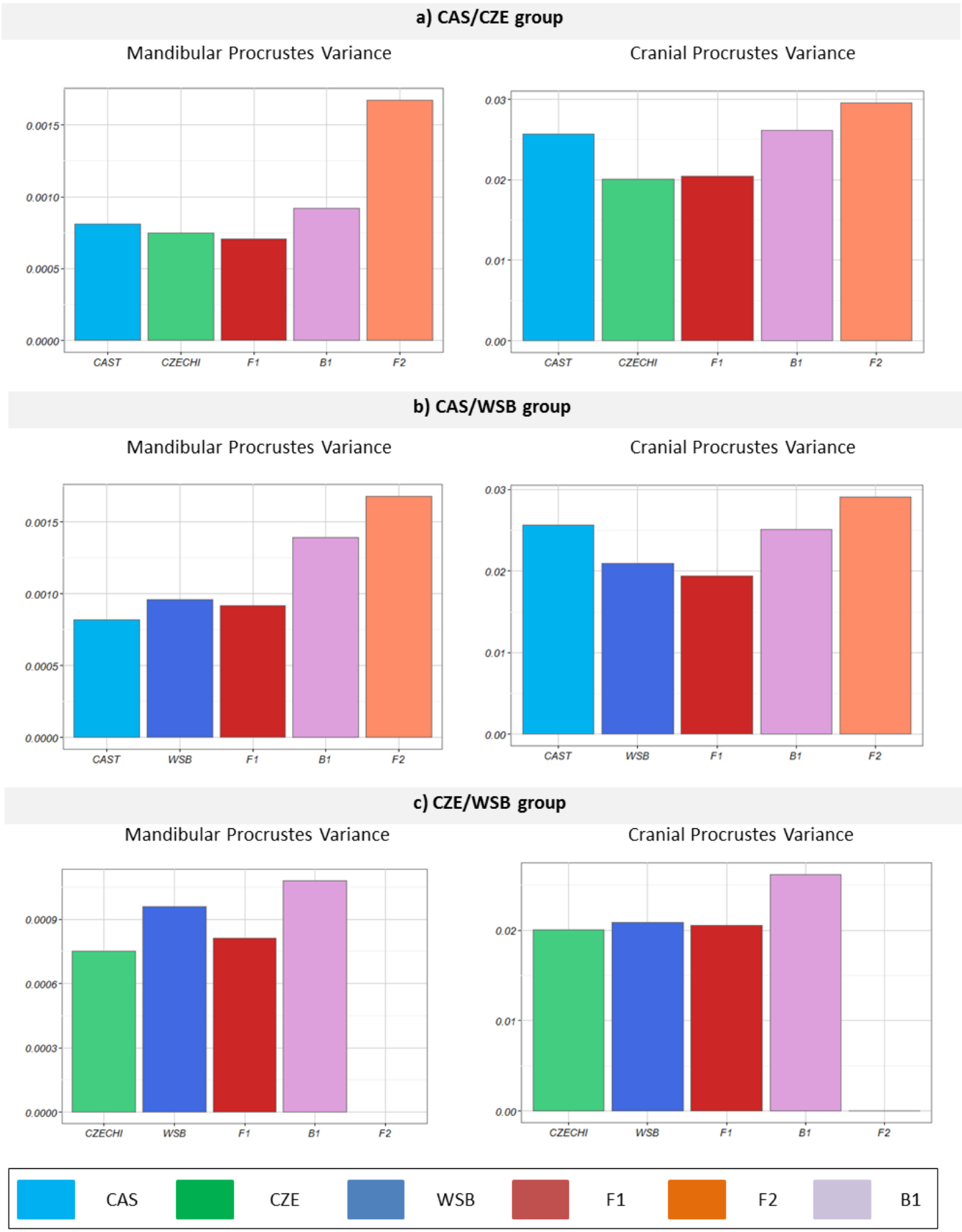


FIGURE 7.3.3. PROCRUSTES VARIANCES FOR THE THE A) CAS/CZE, B) CAS/WSB AND C) CZE/WSB GROUPS, IN BOTH MANDIBLE (LEFT) AND CRANIUM (RIGHT).

Figure 7.3.3 also shows the cranial Procrustes variances of the different strains within each group. There are clear differences between CAS and the other two parent groups, with CAST displaying significantly greater Procrustes variance than CZE and WSB (which are not significantly different from each other). The F1 hybrids have similar or smaller Procrustes variances compared with the parents, being significantly smaller than CAST in the CAS/CZE and CAS/WSB groups (in both cases below p=0.001). The CASxCZE and CZExWSB F1s are slightly larger than CZECHI, but not significantly so. The CZExWSB F1 hybrids are of similar magnitude to WSB, but the CASTxWSB F1 hybrids are slightly smaller (p=0.03).

In the two crosses where there are mutigenerational hybrids, we see comparable Procrustes variances between B1s and the more variable parent (i.e. not significantly different). The F2s, however, have significantly greater Procrustes variances than the parent strains.

CLASSIFICATION

Figure 7.3.4 shows the simple classification of individuals within strains to either the parents or F1 hybrids using Procrustes distances. It is worth noting that these classifications are simple, and neither group is weighted, given that parents and F1 hybrids do not have significantly different variances in these metrics.

If we interpret the proportions in the graphs showing similarities to the mean mandibular Procrustes distances of groups as individuals resembling parent or hybrid form, we see a clear pattern in the parents and F1 hybrids. Over 96% of individuals in each of the parent and F1 hybrid strains more closely resemble the mean Procrustes distance of that strain. In the F2s, the majority of individuals resemble the form of F1 hybrids and one of the parents (CZE – the larger parent - in the CAS/CZE group, and CAS – the smaller parent - in the CAS/WSB group). B1 individuals more closely resemble in shape the F1 hybrid or parent with which they are backcrossed.

Figure 7.3.4 also shows the classification of individuals within strains to either the parents or F1 hybrids using Procrustes distances of cranial shape. The patterns are similar to those seen in the mandible.

If we interpret the proportions in the graphs showing similarities to the mean Procrustes distances of strains as individuals resembling parent or hybrid shape, the pattern of classification is 100% correct (i.e. all parent and F1 individuals are correctly classified). All individuals in each of the parent and F1 hybrid strains more closely resemble the mean Procrustes distance of that strain. In the F2s, the majority of individuals resemble the shape of F1 hybrids. B1 individuals are more likely to closely resemble either the F1 hybrid (particularly seen in the CAS/WSB cross) or parent with which they are backcrossed in shape (as seen in the CAS/CZE cross).

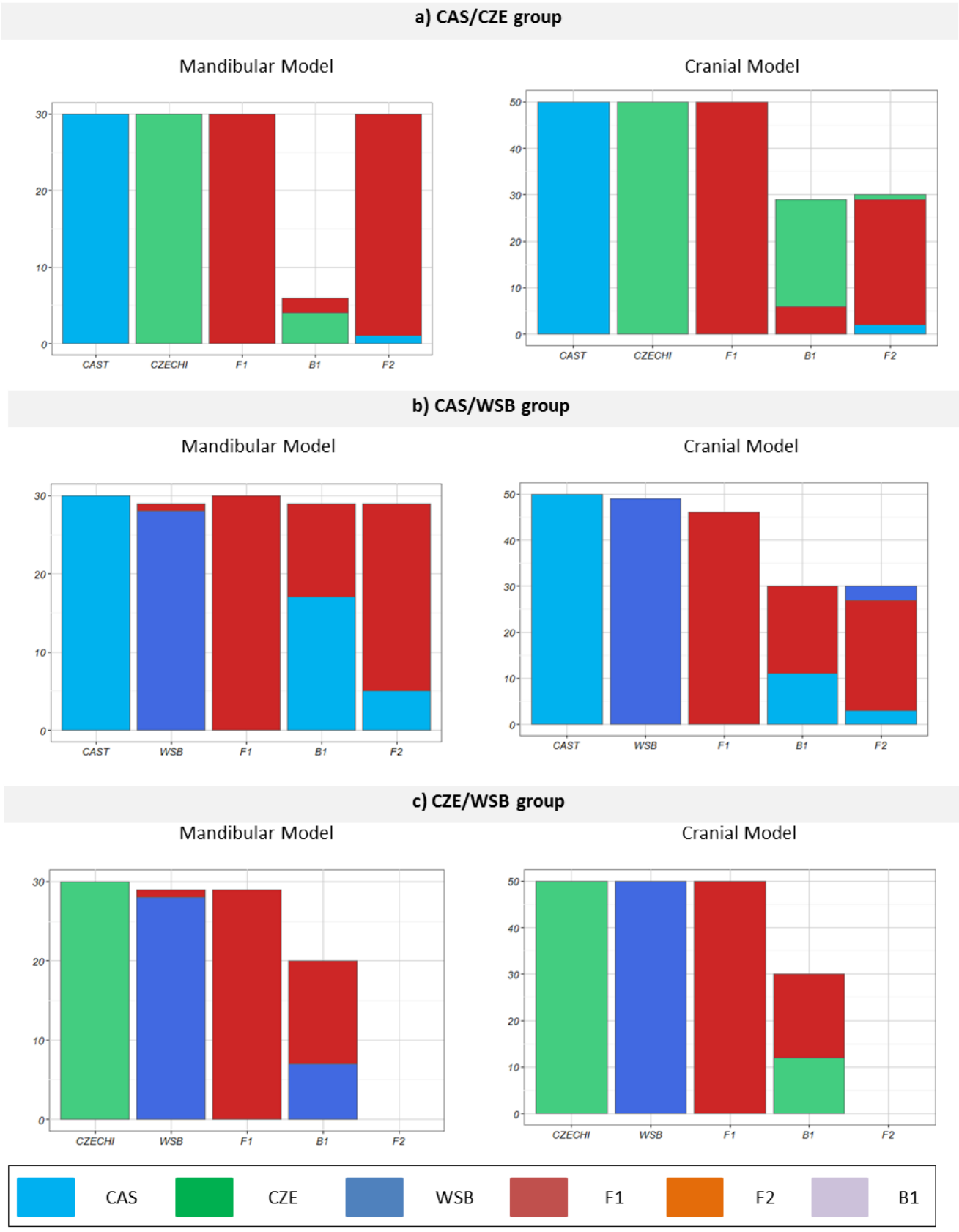


FIGURE 7.3.4. RESULTS OF THE CLASSIFICATION MODEL, AS PERFORMED USING PROCRUSTES DISTANCE FOR THE MANDIBLE (LEFT) AND CRANIUM (RIGHT). INDIVIDUALS IN EACH STRAIN ARE ASSIGNED AS EITHER PARENT OR F1, BASED ON PROXIMITY TO STRAIN MEAN WITHIN THE A) CAS/CZE, B) CAS/WSB AND C) CZE/WSB GROUPS.

PART 4: INTERSPECIFIC CROSSES

A series of analyses similar to what was done for the intra-specific crosses was also performed on the interspecific cross of *Mus musculus* (subsp. *musculus*: WSB) and *Mus spretus* (SPRET).

CRANIAL SIZE: INTERSPECIFIC HYBRIDIZATION

Figure 7.4.1a shows the cranial sizes of the groups used in this study. Among the strains used in the interspecific group, WSB displays the smaller cranial size of the two parents, despite being the largest of the intraspecific hybrids (Figure 7.1.1). SPRET is much larger in cranial size than the *Mus musculus* subspecies. Remarkably, while the sub-specific F1 hybrids are significantly larger than all parent strains, the interspecific F1 hybrids (WSBxSPR) are significantly larger than WSB ($P<0.05$), but not significantly different from SPRET. This may be because hybrid vigour is more pronounced in more closely related groups, or because the parent SPRET is already so much larger in cranial size than the other mice. However, the WSBxSPR F1 hybrid also has smaller cranial size than the intraspecific F1 hybrids as well.

TABLE 7.4.1 TUKEY TEST STATISTICAL MATRIX (LEFT) AND THE RESULTS OF THE ANOVA AND LEVENE'S TEST (RIGHT) FOR COMPARISONS AMONG STRAINS WITHIN THE WSB/SPR GROUP.

WSB/SPR group									
	WSB	**SPRET**	**F1**	**ANOVA**	**Df**	**Sum sq**	**Mean sq**	**F value**	**Pr(>F)**
WSB	0	0	0.045	**Strain**	2	21219	10610	12.09	1.50E-05
SPRET	**29.1**	–	0.096	**Resid**	133	116715	878		
F1	*15.6*	13.5	–	**Levene**	**Df**	**F value**	**Pr(>F)**		
					2	1.8033	0.1688		

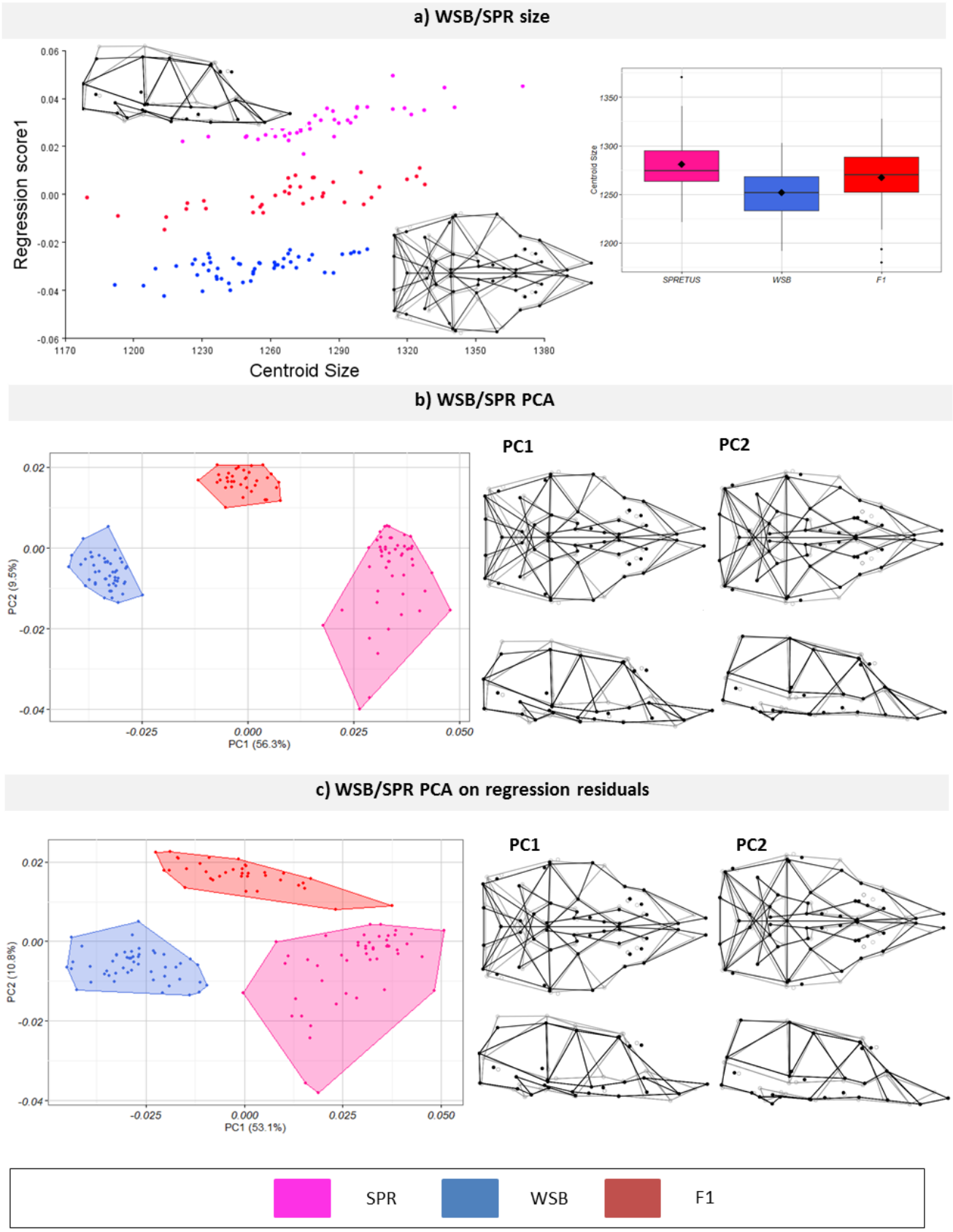
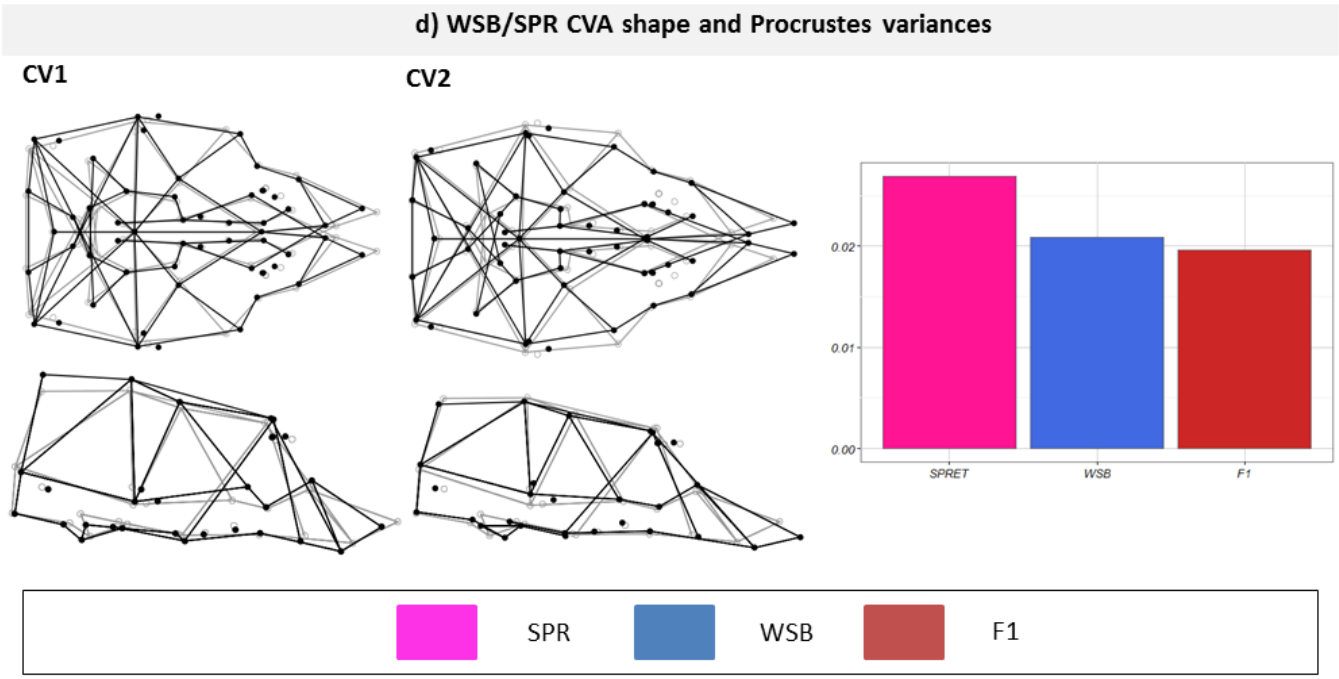


FIGURE 7.4.1. WSBXSPR GROUP CRANIAL RESULTS. THESE INCLUDE ANALYSES ON A) SIZE AND ALLOMETRY, B) PRINCIPAL COMPONENTS ANALYSIS ON PROCRUSTES ALIGNED COORDINATES, C) PRINCIPAL COMPONENTS ANALYSIS ON REGRESSION RESIDUALS AND D) CANONICAL VARIATES ANALYSIS AND PROCRUSTES VARIANCES.



Allometry

In the regression (Figure 7.4.1a), the interspecific strains show similar parallel trajectories with respect to regression. Size accounts for 12.4% of the total shape differences and is therefore significant ($p<0.0001$). However, many of the size related shape changes seem to occur within each strain, and size appears not to be as defining for size differences between strains. In general, however, some size-related shape may explain some of the features seen in the PCA (PC1) and CVA (CV1), such as the elongation of the snout.

Principal Components Analysis

The pattern seen in the PCA of PC1 vs PC2 is similar to that seen in the intraspecific crosses (Figure 7.4.1b): the greatest variance lies across PC1, which mainly highlights differences between parents, with the hybrids lying intermediate to the parent strains. However, in this PCA, PC1 accounts for 56.3% of the total variance, which is a far greater proportion than those seen in the intraspecific hybrids, indicating very large shape differences among the parents. Shape change along this PC largely shows a lengthening of the snout and more posterior position of temporal landmarks in SPRET compared with WSB. Along PC2, there is also a similar pattern to that seen in the intraspecific crosses: the separation between the F1 hybrids and the parents. However, PC2 only accounts for 9.5% of the total

variance. Shape change along PC2 seems to resemble that noted in the intraspecific crosses: elongation in the snout of the hybrids and a thinning of the face.

PCA on Regression Residuals

Using the regression residuals to form a PCA (Figure 7.4.1c), we can see that there is a lot more overlap between groups along PC1 when size is taken into account. However, the relative positioning of the different strains remains the same. PC1 (53.1% of the variance) shows separation between the two parents, but the hybrids, which are intermediate, overlap more closely with both parents. Shape change along this axis appears similar to that in the original PCA: SPRET displays a longer snout and more posterior landmarks in the anterior part of the zygomatic. Along PC2 (10.8%), the F1 hybrids are still separate from the parents, with no overlap of parents and hybrids. The shape change here also resembles that seen in the former PCA: hybrids have longer snouts and thinner faces.

Canonical Variate Analysis

A CVA on the three strains in the WSB/SPR group shows greatest separation between the two parents (90.5% in CV1) with the F1 hybrids as intermediate, and F1s separated from parents along CV2 (9.5% of differences between groups). In this scenario, the Mahalanobis and Procrustes distances between groups are all significantly different. In this scenario, the greatest distances are between the parents (53 Mahalanobis; 0.067 Procrustes), and the hybrids of similar shape distance to both parents (33.9/0.043 to SPRET, and 28.6/0.041 to WSB). In Figure 7.4.1d, the shape differences along CV1 (between parents) show a thinner and longer anterior face, and relatively short neurocranium in SPRET. Along CV2, the hybrids have thinner heads and more projecting faces compared with either of the parents.

PART 5: INTEGRATION AND MODULARITY

MODULARITY

Table 7.5.1 shows the RV coefficients for each strain for three comparisons: (1) between the facial and neurocranial components of the cranium, (2) between the facial, neurocranial and basicranial regions of the cranium (subdividing the neurocranial region in the initial analysis), and (3) between the anterior and posterior mandibular regions. This table also includes the proportion of randomly selected modules (10 000 were taken in total for each analysis) which had lower integration than those hypothesized. A low proportion value is therefore supportive of relative modularity of the hypothesized regions. A high RV coefficient is similar to an R^2 value; a high value supports strong integration of components. Figures 7.5.1-7.5.3 shows the distribution of RV coefficients for the 10 000 randomly selected landmark partitions for each analysis for each hypothesis.

TABLE 7.5.1. RV COEFFICIENTS AND PROPORTIONS OF RANDOM MODULES WITH LOWER INTEGRATION THAN THE HYPOTHESIZED.

	Face / Basicranium-neurocranium		**Face / Basicranium / Neurocranium**		**Anterior / Posterior Mandible**	
	RV coefficient	**Proportion**	**RV coefficient**	**Proportion**	**RV coefficient**	**Proportion**
CAST	0.5399	0.0058	0.4292	0.0228	0.4329	0.2433
CZECHI	0.3881	0.0128	0.3064	0.1316	0.3405	0.0093
WSB	0.4810	0.0070	0.3977	0.2477	0.4576	0.0232
CASxCZE F1	0.4793	0.0112	0.4527	0.8253	0.4224	0.1207
CASxWSB F1	0.5016	0.0244	0.3833	0.0413	0.5321	0.4360
CZExWSB F1	0.5008	0.0339	0.3739	0.0517	0.4434	0.3349
CASxCZE F2	0.4219	**0.0000**	0.4105	0.5797	0.4076	0.1897
CASxWSB F2	0.5339	0.0029	0.4502	0.0116	0.4047	0.0693
(CASxCZE)xCZE	0.5017	0.0071	0.4485	0.4034	0.8059	0.4139
(CASxWSB)xCAS	0.4791	0.0046	0.4113	0.1424	0.3665	0.1342
(CZExWSB)xWSB	_	_	_	_	0.4616	0.2523
SPRET	0.7367	0.0719	0.6816	0.3900	_	_
WSBxSPR F1	0.6795	0.3237	0.5697	0.5752	_	_

Figure 7.5.1 shows the distribution of two partitions of the cranium, the arrows showing the RV coefficients of the hypothesis that the face and whole neurocranium are modules for each strain (see also Table 7.5.1). Modules in mice crania are variably integrated among the mouse strains. Among the parents, modularity between the face and neurocranium is generally supported (Proportion<0.05 for the three *Mus musculus* strains, and 0.07 for the SPRET strain). However, the RV coefficients for the hypothesized modules (and the distribution of RV coefficients for the random partitions), are variable among strains. Integration is higher in SPRET than in the *Mus musculus* strains. Among the *Mus musculus* strains, CZECHI appears to have weaker integration than the other strain (RV=0.388 for the hypothesized module). Among the intraspecific F1 hybrids, the hypothesis of modularity in the face and neurocranium is supported (Proportion<0.05), but is rejected in the interspecific SPRxWSB F1 hybrid. Despite this, integration in the cranium as a whole is far higher in the SPRxWSB F1 hybrid (RV= 0.68) than the intraspecific hybrids (RV is between 0.48 and 0.5). The CZExWSB F1 hybrid exhibits an RV coefficient higher than the two parents (RV = 0.5), and the CASxWSB and CASxCZE F1 hybrids exhibit intermediate RV coefficients. Similarly, among the F2s and backcrosses, integration seems moderate, albeit variable. CASxCZE F2 has the weaker cranial integration at RV=0.42, although it is still higher than the CZECHI parent. CASxWSB has the higher RV coefficient (0.53), comparable with CAST (0.54). In the backcrosses, CASxCZE_CAS has a higher RV coefficient (0.501) than the CASxCZE F1 hybrid (0.48) and CZECHI (0.388), but lower than CAST (0.54).

As seen in Table 7.5.1 and Figure 7.5.2, only three strains exhibit modularity between the three hypothesized modules of the cranium: CAST, CASxCZE F1 and CASxWSB F2. RV coefficients are higher in SPRET and the interspecific cross (similar to that seen in the two-partitioned cranial hypothesis), yet the hypothesis of modularity is rejected. Modularity in these three partitions is not supported in the majority of these strains, possibly due to high integration between the neurocranium and basicranium.

Figure 7.5.3 shows the distributions of RV coefficients for two partitions of the mandible in the strains. In this analysis, integration in the cranium is moderate (most of the distributions centre between RV= 0.4 and 0.6). The exception to this is the CASxCZE_CZE backcross, where the RV for the hypothesis of modularity between the anterior and posterior mandible is 0.81. (This, however, may also be a function of small sample size, where n=6). Modularity between the anterior and posterior mandible is only supported in the parents, CZECHI and WSB (Prop<0.05) and possible the CASxWSB F2 hybrids (Prop=0.07).

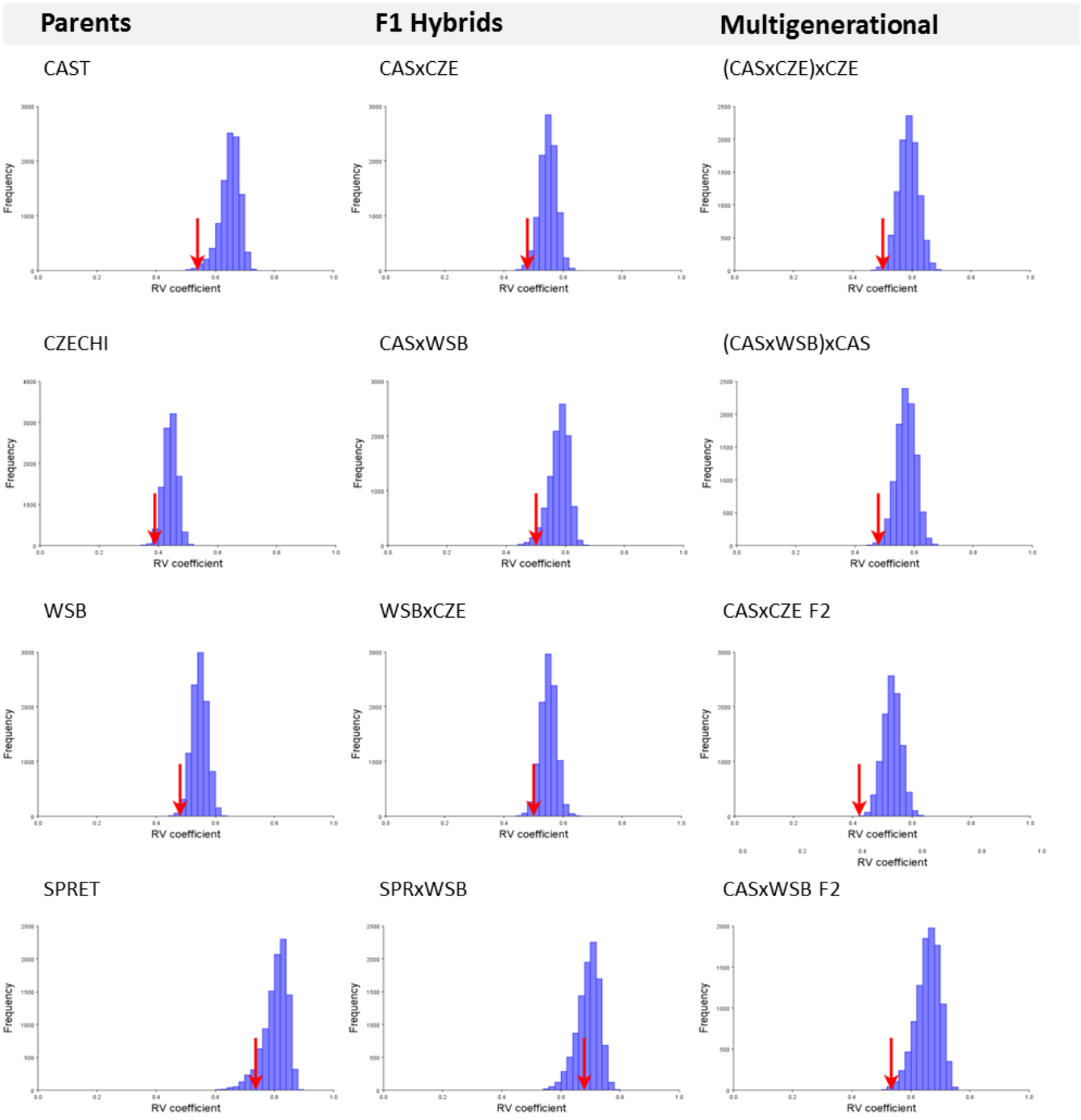


FIGURE 7.5.1. MODULARITY TESTS ON THE CRANIUM (TWO PARTS) FOR EACH STRAIN. EACH GRAPH SHOWS THE RV COEFFICIENTS FOR THE SUBDIVISION OF LANDMARKS INTO THE FACE AND NEUROCRANIUM, AND A DISTRIBUTION OF RV COEFFICIENTS FOR 10 000 ALTERNATIVE PARTITIONS OF LANDMARKS.

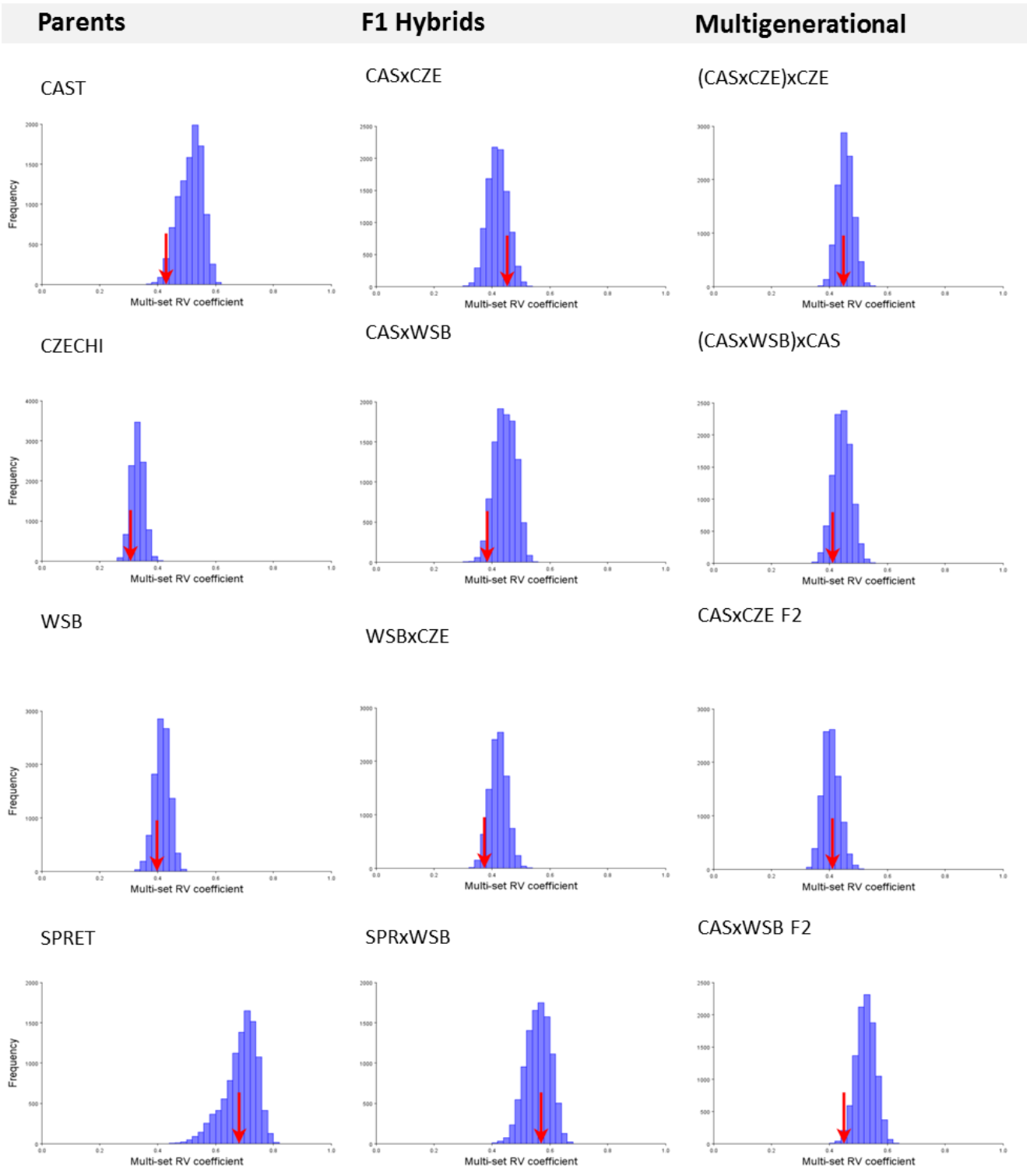


FIGURE 7.5.2. MODULARITY TESTS ON THE CRANIUM (THREE PARTS) FOR EACH STRAIN. EACH GRAPH SHOWS THE RV COEFFICIENTS FOR THE SUBDIVISION OF LANDMARKS INTO THE FACE, BASICRANIUM AND NEUROCRANIUM, AND A DISTRIBUTION OF RV COEFFICIENTS FOR 10 000 ALTERNATIVE PARTITIONS OF LANDMARKS.

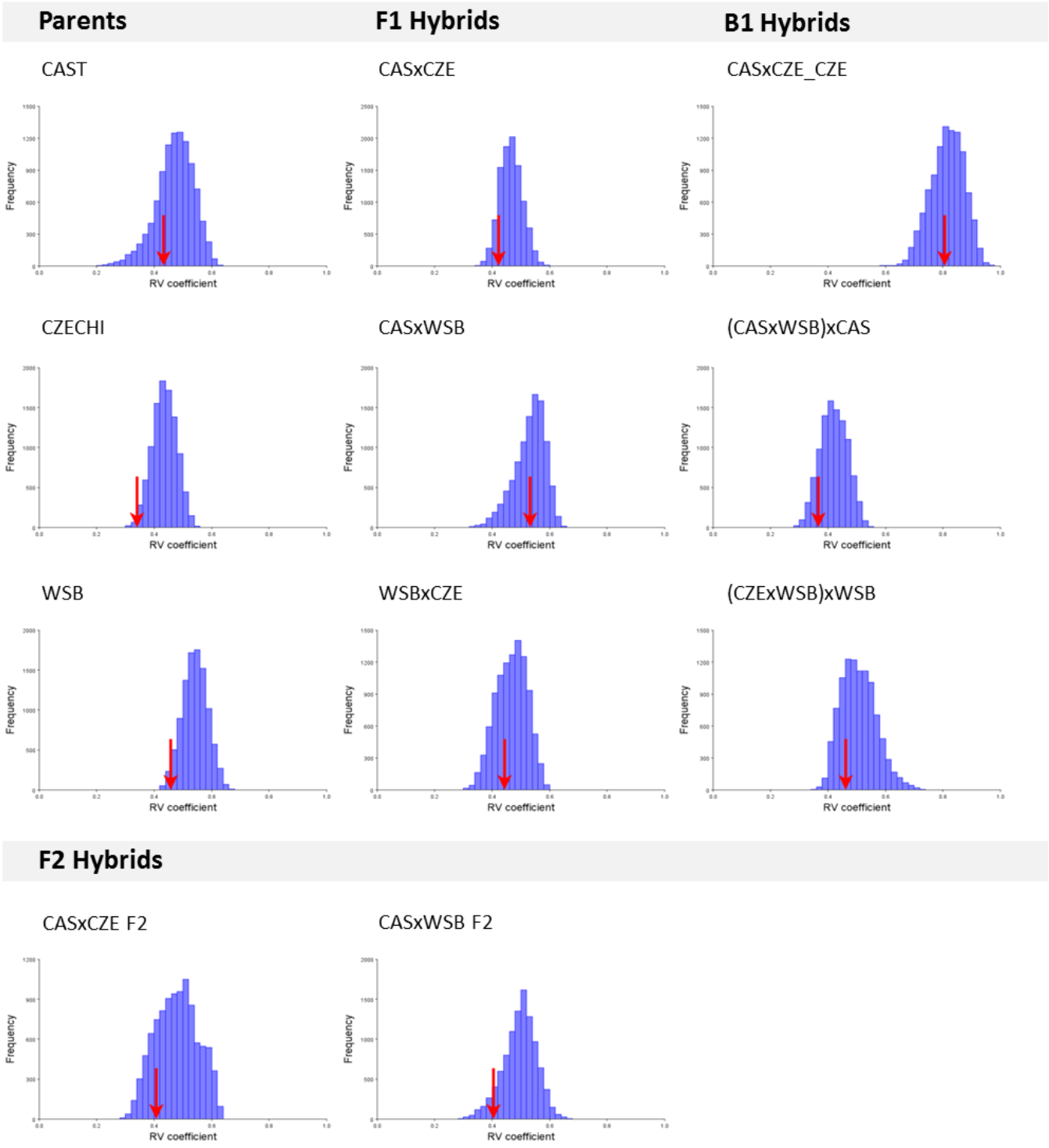


FIGURE 7.5.3. MODULARITY TESTS ON THE MANDIBLE FOR EACH STRAIN. EACH GRAPH SHOWS THE RV COEFFICIENTS FOR THE SUBDIVISION OF LANDMARKS INTO THE ANTERIOR AND POSTERIOR MANDIBLE, AND A DISTRIBUTION OF RV COEFFICIENTS FOR 10 000 ALTERNATIVE PARTITIONS OF LANDMARKS.

COVARIANCE CORRELATIONS

Although there is little support for modularity in the anterior and posterior regions of the mandible for most strains, separating the mandible into regions reduces the landmarks (i.e. variables) for correlation tests, and is important in analysing correlations among covariance matrices of different strains. Table 7.5.2 shows matrices of the correlations between strains in the anterior and posterior mandible. Correlations among parents in the anterior mandible are high: CAST to CZECHI (0.667), CAST to WSB (0.779) and CZECHI to WSB (0.663).

However, the correlations between parents and hybrids are higher than among their respective parents (the CASTxWSB F1 hybrid is correlated with WSB at 0.92). In the CAS/CZE group, most correlations were significant at 0.01, except for CZECHI to the (CASxCZE)xCZE backcross (matrix correlation=0.456). CZECHI is also not significant at P=0.001 when correlated with the CASxCZE F2. The CASxCZE F2/F1 comparison yields the highest correlation at 0.84 (albeit comparable with the comparison of F2 to CAS-0.82- and F1 to CAS-0.81). Furthermore, correlations between the B1 and other strains are moderate (0.456 compared with CZECHI to 0.623 compared with CAST; F1 and F2 hybrids in between). In the CAS/WSB group, the F2 hybrid is highly correlated with F1 (0.889). All comparisons among strains in the CAS/WSB group are significant at P=0.001, although it is worth noting that correlations between the B1s and other strains were high, but not significant at the conservative P<0.0001.In the CZE/WSB group, the F1 hybrid is highly correlated with the parents (CZE=0.796 and WSB=0.878; significant at P<0.0001). The correlation among the covariance matrix for the backcrosses to parents and F1 strains are all non-significant (even at a liberal P<0.05).

Correlations among parents in the posterior mandible are variable: CAST to CZECHI and CZECHI to WSB are significantly correlated (0.735 and 0.676, respectively; P<0.0001); but CAST and WSB are only moderately correlated (0.574; P=0.027). In the CAS/CZE group, the F2 hybrids are not significantly correlated to the other strains at P=0.001. The B1s are not significantly correlated with CAS (0.333; p=0.178), but are correlated with CZE and the F1 (at a liberal P<0.05) and F2 (P=0.001). For the CAS/WSB group, the F1s and F2s, and B1s and CAS, are highly correlated (0.719 and 0.711, respectively; significant at P<0.0001). The other comparisons between hybrids and parent strains in this group are all significantly correlated at p<0.01. In the CZE/WSB group, the F1s and B1s are highly correlated with CZE (0.754 and 0.699 respectively, P<0.0001), and moderately correlated with WSB (0.63 and 0.603; P<0.01). The F1s and B1s are highly correlated with each other as well (0.666; P<0.0001).

TABLE 7.5.2. CORRELATIONS OF THE COVARIANCE MATICES BETWEEN DIFFERENT GROUPS FOR THE ANTERIOR (LEFT) AND POSTERIOR (RIGHT) MANDIBULAR MODULES (IF P< 0.05 UNDERLINED, IF P<0.001 THEN ITALICS, IF P<0.0001 THEN BOLD).

	Anterior mandible						Posterior mandible				
					CAS/CZE						
	CAS	**CZE**	**F1**	**F2**	**B1**		**CAS**	**CZE**	**F1**	**F2**	**B1**
CAS	–					**CAS**	–				
CZE	0.6667	–				**CZE**	**0.7349**	–			
F1	*0.8127*	**0.7138**	–			**F1**	*0.7084*	**0.6506**	–		
F2	*0.8242*	0.6453	**0.8395**	–		**F2**	0.6414	0.6324	0.6354	–	
B1	*0.6229*	0.4558	*0.5965*	*0.5798*	–	**B1**	0.3327	0.3941	0.3904	0.4444	–
					CAS/WSB						
	CAS	**WSB**	**F1**	**F2**	**B1**		**CAS**	**WSB**	**F1**	**F2**	**B1**
CAS	–					**CAS**	–				
WSB	**0.7797**	–				**WSB**	0.5736	–			
F1	**0.8054**	**0.9237**	–			**F1**	0.6211	0.6093	–		
F2	**0.751**	**0.8464**	**0.8890**	–		**F2**	0.6070	*0.6655*	**0.7195**	–	
B1	*0.8603*	*0.8568*	**0.8426**	*0.8391*	–	**B1**	**0.7105**	0.6170	*0.6092*	*0.6819*	–
					CZE/WSB						
	CZE	**WSB**	**F1**	**B1**			**CZE**	**WSB**	**F1**	**B1**	
CZE	–					**CZE**	–				
WSB	0.6627	–				**WSB**	**0.6758**	–			
F1	**0.7962**	**0.8778**	–			**F1**	**0.7537**	0.6299	–		
B1	0.5529	0.5611	0.5907	–		**B1**	**0.6992**	0.6035	**0.6659**	–	

In order to reduce the number of landmarks per analysis, and because 10 of the 12 strains analysed supported the hypothesis of modularity, covariance matrices for the facial-palatal and neurocranial modules were calculated separately. Matrices showing the correlations between covariance matrices of different strains are in Table 7.5.3. Parent strains are significantly correlated in the facial-palatal region.

In the facio-palatal region of the cranium, all intraspecific strains and hybrids are significantly correlated. However, it is clear that some correlations are higher than others. Correlations between CAS and CZE, and CAS and WSB, are low (0.37 and 0.385 respectively). CZE and WSB covariance matrices, however, are highly correlated (0.73). F1 and F2 hybrids in the CAS/CZE and CAS/WSB groups appear to be more greatly correlated with one parent than the other (CZE and WSB, respectively). CZExWSB hybrids are also better correlated with WSB (0.72) than CZE (0.66), but the difference in R^2 value is smaller in this comparison. Backcrosses appear to be more greatly correlated in covariance structure with F1 hybrids or the parents with which they are backcrossed.

In the neurocranium, all intraspecific comparisons are significantly correlated in covariance structure ($p<0.001$). Similar to what is seen in the facio-palatal comparisons, F1 and F2 hybrids more closely resemble one parent than the other: CZE in the CAS/CZE group, and WSB in the CAS/WSB group. B1s appear best correlated with F2s in this module.

The interspecific strains (WSB and SPRET) are poorly correlated in the faciopalatal (0.24) and neurocranial (0.39) modules. The F1s are much more highly correlated with the WSB covariance matrices than with SPRET.

TABLE 7.5.3 CORRELATIONS OF COVARIANCE MATRICES BETWEEN GROUPS FOR THE FACIO-PALATAL (LEFT) AND NEUROCRANIUM (RIGHT) MODULES (IF P< 0.05 UNDERLINED, IF P<0.001 THEN ITALICS, IF P<0.0001 THEN BOLD).

Facio-palatal						Neurocranium					
CAS/CZE											
	CAS	**CZE**	**F1**	**F2**	**B1**		**CAS**	**CZE**	**F1**	**F2**	**B1**
CAS	–					**CAS**	–				
CZEC	**0.3741**	–				**CZE**	**0.3999**	–			
F1	**0.3998**	**0.5896**	–			**F1**	**0.3511**	**0.6410**	–		
F2	**0.6295**	**0.4948**	**0.5121**	–		**F2**	**0.3424**	**0.5875**	**0.6055**	–	
B1	**0.3787**	**0.6632**	**0.6070**	**0.4517**	–	**B1**	*0.3259*	**0.6282**	**0.6021**	**0.6463**	–
CAS/WSB											
	CAS	**WSB**	**F1**	**F2**	**B1**		**CAS**	**WSB**	**F1**	**F2**	**B1**
CAS	–					**CAS**	–				
WSB	**0.3850**	–				**WSB**	**0.3407**	–			
F1	**0.4235**	**0.7180**	–			**F1**	**0.3411**	**0.6525**	–		
F2	**0.3548**	**0.5671**	**0.6323**	–		**F2**	**0.3577**	**0.6320**	**0.7166**	–	
B1	**0.6099**	**0.4899**	**0.4656**	**0.4615**	–	**B1**	**0.3399**	**0.5932**	**0.6665**	**0.7067**	–
CZE/WSB											
	CZE	**WSB**	**F1**				**CZE**	**WSB**	**F1**	**B1**	
CZE	–					**CZE**	–				
WSB	**0.7259**	–				**WSB**	**0.6746**	–			
F1	**0.6636**	**0.7183**	–			**F1**	**0.7193**	**0.6967**	–		
WSB/SPR											
	SPR	**WSB**	**F1**				**SPR**	**WSB**	**F1**		
SPR	–					**SPR**	–				
WSB	<u>0.2421</u>	–				**WSB**	**0.3903**	–			
F1	*0.2593*	**0.6172**	–			**F1**	**0.3887**	**0.5283**	–		

Purpose: To quantify the non-metric trait variation in the mice: parents, F1 hybrids and multigenerational recombinants.

CHAPTER 8

NON-METRIC TRAIT VARIATION IN HYBRIDS AND RECOMBINANTS

FREQUENCY OF NON-METRIC TRAIT OBSERVATIONS

All recorded non-metric traits are reported in Appendix 2. In the appendix, the absolute numbers (not proportions) of specimens are reported, with the trait listed in each column. Also recorded is whether features are seen bilaterally or unilaterally where relevant.

DENTAL VARIATION

No supernumerary teeth were detected in any of the samples. Two teeth were recorded as rotated in SPRET, and two teeth were recorded as "skew" (tilted towards the M2s) in the backcross,

(WSBxCZE)xWSB. "Pegging" and M3 reduction was recorded in several individuals: two CAS, one CASxWSB F2, one (CASxCZE)xCZE and one (WSBxCZE)xWSB (Table 8.1).

TABLE 8.1. PROPORTIONS OF ROTATION, REDUCTION, MISSING TEETH AND WORN DENTAL PATTERNS (BOLD= SIGNIFICANT AT 0.05; UNDERLINED= SIGNIFICANT AT 0.00001).

Strain	n	Rotated /skew	Peg/ reduced	Missing/ Broken/ worn together	Missing	Broken teeth	Teeth too worn		
		M3s	M3s	All	M3s	M1s	M1s	M2s	M3s
CAS	50	0	0.04	0.14	0.14	0.06	0	0.02	0
WSB	50	0	0	0.02	0	0	0.02	0.02	0.02
CZE	50	0	0	0.1	0.1	0	0	0	0
SPR	50	0.04	0	0.56	0.3	0	0.28	0.28	0.36
CASxWSB	50	0	0	0	0	0	0	0	0
CASxCZE	50	0	0	0	0	0	0	0	0
WSBxCZE	50	0	0	0	0	0	0	0	0
SPRxWSB	36	0	0	0	0	0	0	0	0
CASxWSB_F2	50	0	0.02	0.02	0.02	0	0	0	0
CASxCZE_F2	50	0	0	0.04	0.04	0	0	0	0
(CASxWSB)xCAS	50	0	0	0	0	0	0	0	0
(CASxCZE)xCZE	48	0	0.02	0	0	0	0	0	0
(WSBxCZE)xWSB	50	0.04	0.02	0.1	0	0	0.04	0.04	0.1
Parental	200	0.01	0.01	0.205	0.14	0.015	0.075	0.08	0.095
Hybrid	434	0.005	0.007	0.018	0.007	0	0	0	0.012
X2		0.634	0.166	**66.8**	**52.1**	**6.54**	**33.3**	**35.6**	**26.2**
F	332	0.006	0.009	0.063	0.039	0.006	0.024	0.024	0.033
M	302	0.007	0.007	0.093	0.059	0.003	0.026	0.03	0.043
X2		0.01	0.12	1.92	1.42	0.25	0.04	0.197	0.43
Parental F	93	0	0	0.161	0.11	0.02	0.075	0.075	0.086
Hybrid F	239	0.008	0.013	0.025	0.013	0	0.004	0.004	0.013
X2		0.78	1.18	**20.96**	**18.9**	**5.17**	**14.39**	**14.39**	**11.28**
Parental M	106	0.019	0.019	0.245	0.17	0.009	0.075	0.075	0.104
Hybrid M	196	0	0	0.01	0	0	0.005	0.005	0.01
X2		**3.72**	**3.72**	**45.19**	**35.39**	1.86	**11.78**	**11.78**	**14.62**

Table 8.1 shows the incidents recorded in each strain for molar rotation, reduction or lifestyle effects (dental loss, wear or breaking). Missing teeth were recorded in parents (seven CAS individuals, five

CZE individuals, and 15 SPRETUS individuals), although it is not always clear as to whether this is congenital (a potential factor in inbreeding) or due to erratic chewing and/or other signs of stress behaviour. In many incidents, it is likely the latter scenario since there appears to be a lot of alveolar space behind the M2s. Reports that parent strains were behaviourally "wild" and energetic, with clear indications of heightened stress levels, relative to both other laboratory mice, and even their recombinants, also support the latter scenario (Robyn Humphreys and Vanessa De Freitas, pers. comm.). Similarly, dental wear occurred in far greater numbers in the parents (particularly SPRET; 21 individuals, but also in CAS–three individuals, and WSB–one individual), although it was also recorded in (WSBxCZE)xWSB (five individuals). Teeth appeared "broken" or bifurcated in three CAS individuals. These traits were also evaluated for differences between males and females, but tests indicate that there are no significant differences between pooled males and females. However, males, on average, had far greater proportions of missing M3s, especially considering the pooled parent samples (10 females, 18 males). But, among the hybrids, the only three with missing M3s were female.

TABLE 8.2. MOLAR CUSP NUMBER VARIATION.

Strain	n for M1s and M2s	Cusps M1s	Cusps M1s	Cusps M2s	n for M3s	Cusps M3s	Cusps M3s
		<6	>6	>4		<3	>3
CAS	50	0.02	0	0.42	45	0.044	0
WSB	50	0.04	0.04	0.52	49	0	0.1
CZE	50	0.1	0.02	0.56	48	0	0.021
SPR	50	0	0	0.22	26	0.077	0.35
CASxWSB	50	0	0	0.86	50	0	0
CASxCZE	50	0	0	0.72	50	0	0
WSBxCZE	50	0.04	0	0.82	50	0	0
SPRxWSB	36	0	0	0.31	36	0	0.06
CASxWSB_F2	50	0	0.02	0.64	50	0.04	0.06
CASxCZE_F2	50	0.02	0.02	0.7	49	0.061	0.02
(CASxWSB)xCAS	50	0	0	0.92	50	0.04	0.04
(CASxCZE)xCZE	48	0	0	0.35	48	0	0.104
(WSBxCZE)xWSB	50	0	0	0.7	47	0.04	0.085
Parental	200	0.04	0.015	0.43		0.02	0.075
Hybrid	434	0.007	0.005	0.682		0.021	0.039
X2		**8.79**	1.89	**36.3**		0.047	**5.9**

Molar cusp number (Table 8.2) was not highly variable for M1s: only 11 individuals had M1s with fewer than 6 cusps (eight parents–one CAST, two WSB and five SPRET; and three recombinants–two

WSBxCZE F1s and one CASxCZE F2), although it was significantly different among Parents and Hybrids (Table 8.2). Only five individuals had seven cusps (two WSB, one CZE, one CAS WSB F2, one CASXCZE F2), and these frequencies were not significantly different among groups. Lower M2s were more variable, with around half of those scored displaying five cusps (not always bilaterally). It is important to note that wear (particularly in SPRET) means that these features are likely underscored in most specimens. The number of specimens with more than four cusps is significantly larger in proportion in recombinants (68%) than parents (43%), even when excluding individuals with heavily worn teeth. These proportions are particularly high in the intraspecific hybrids (especially CASxWSB F1—86%, and (CASxWSB)xCAS B1—92%), as compared to the interspecific SPRxWSB cross (22%).

When looking at M3s, it is important to note that in some groups (particularly parents: CAS, CZE and SPRET) M3s are missing, and this needs to be taken into consideration in calculating frequencies. A handful of individuals have fewer than three cusps (two CAS, two SPRET, one CASxWSB F2, two CASxCZE F2, one CASxWSB_CZE and two (WSBxCZE)xWSB individuals). These teeth were typically smaller. This was not observed in any of the F1 hybrids. Teeth with four cusps were also observed (although none in CAST or the intraspecific hybrids). Nine individuals have four cusped M3s (six express this bilateral). It also appears that the occlusal surface is generally shallow in this strain, even for those with minimal wear. Many individuals had missing or worn teeth (total 50 M3s), and could not be included in these observations (30% of teeth, 37.5% individuals).

FUSION

For sutural fusion, the following traits were not seen in the dataset: squamosal-frontal, squamosal-parietal and post-tympanic hook fusion. This may be due to these features being difficult to observe on scans with some certainty. Table 8.3 shows the numbers of individuals observed with fused cranial elements. Nasal fusion was only observed in SPRET, at a high frequency (18 individuals; 36%). Basisphenoid-presphenoid fusion was observed in WSB (15 individuals) and in one WSBxCZE individual. Basisphenoid-basioccipital fusion was observed in two WSB and one CAS individual. Dorsal-frontal fusion was observed in four SPRET and one CASxCZE F1 individual. These features are significantly different among parents and hybrids, although these differences are concentrated in certain strains (as above and shown in Table 8.3).

TABLE 8.3. FUSION IN CRANIAL TRAITS.

Strain	n	Nasal fusion	Basisphenoid-basioccipital	Basisphenoid-presphenoid fusion	Dorsal frontal fusion
CAS	50	0	0.02	0	0
WSB	50	0	0.04	0.3	0
CZE	50	0	0	0	0
SPR	50	0.36	0	0	0.08
CASxWSB	50	0	0	0	0.02
CASxCZE	50	0	0	0	0
WSBxCZE	50	0	0	0.02	0
SPRxWSB	36	0	0	0	0
CASxWSB_F2	50	0	0	0	0
CASxCZE_F2	50	0	0	0	0
(CASxWSB)xCAS	50	0	0	0	0
(CASxCZE)xCZE	48	0	0	0	0
(WSBxCZE)xWSB	50	0	0	0	0
Parental	200	0.09	0.015	0.075	0.02
Hybrid	434	0	0	0.002	0.002
X2		**40.2**	**6.5**	**29.4**	**5.5**

EXTRA SUTURES

Extra sutures were not observed on the following cranial bones: premaxilla, frontal and squamosal bones. Similar to what was seen for sutural fusion, it is not possible to rule out the possibility that this is due to the bones being particularly thin, and therefore difficult to visualise on scans. However, large parts of the premaxilla and frontal bones were clearly visible, and most anomalous sutures should have been clearly visible.

Only one individual exhibited nasal sutures (CASxCZE F2), and one exhibited maxillary sutures (SPRxWSB F1). In both incidents, this may be a bone fracture, rather than a clear sutural anomaly. However, the maxillary feature does not only occur along weak points of the bone, and therefore could be a congenital anomaly (Table 8.4, Figure 8.1).

TABLE 8.4. EXTRA-SUTURAL ANOMALIES WITHIN CRANIAL BONES.

Strain	n	Nasal	Maxilla	Zygomatic	Parietal	Interparietal	Occipital
CAS	50	0	0	0.12	0	0	0.94
WSB	50	0	0	0	0.04	0	0
CZE	50	0	0	0	0	0	0
SPR	50	0	0	0.04	0	0	0.04
CASxWSB	50	0	0	0.02	0	0	0
CASxCZE	50	0	0	0	0	0	0.02
WSBxCZE	50	0	0	0	0	0.02	0
SPRxWSB	36	0	0.03	0.61	0	0	0
CASxWSB_F2	50	0	0	0.12	0	0	0.16
CASxCZE_F2	50	0.02	0	0.16	0	0	0.06
(CASxWSB)xCAS	50	0	0	0.12	0	0	0.44
(CASxCZE)xCZE	48	0	0	0.042	0.021	0	0
(WSBxCZE)xWSB	50	0	0	0.1	0	0	0
Parental	200	0	0	0.04	0.01	0	0.245
Hybrid	434	0.002	0.002	0.115	0.002	0.002	0.078
X2		0.461558	0.461558	**9.31707**	1.721757	0.461558	**33.4223331**

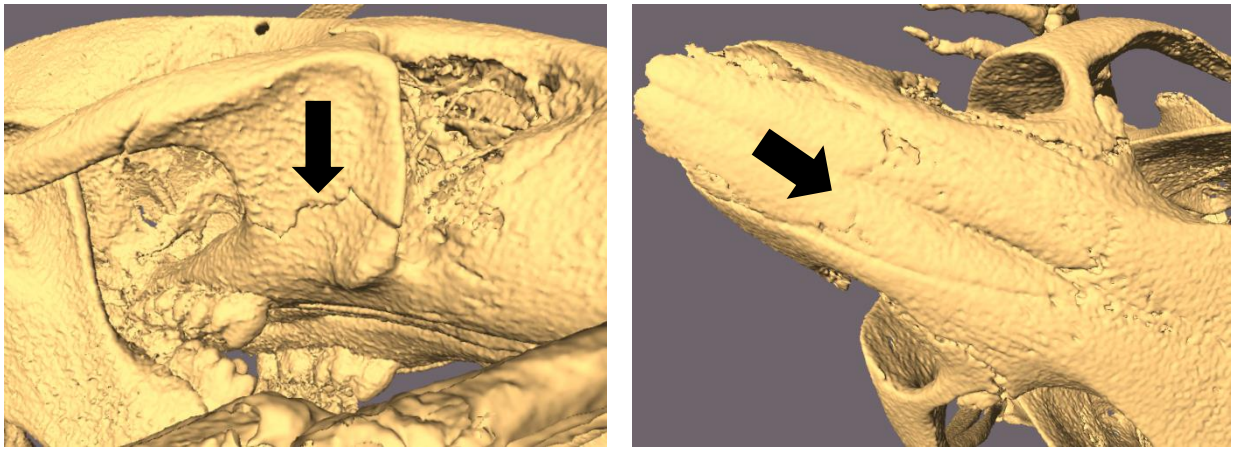


FIGURE 8.1. SPRXWSB35 WITH POTENTIAL EXTRA MAXILLARY SUTURE (LEFT) AND CASXCZE_F2 30 WITH POTENTIAL NASAL SUTURES (RIGHT).

The most common sutural anomaly occurred along the zygomatic bone (n=58; 9%). Often the sutures ran anterio-posteriorly, and were more common on the left zygomatic bone (41 individuals expressed this trait on the left side only, 14 bilaterally and three on the right only). This feature was also more frequently observed in hybrids and recombinants (n=50; 11.5%) than in parents (n=8; 5%). Among

parents, this feature was only observed in CAS (n=6) and SPRET (n=2). Among intraspecific F1 hybrids, only one individual (CASxWSB) expressed this trait. In the interspecific F1 hybrids, 22 individuals expressed an extra zygomatic suture (44%). Among the intraspecific F2s, CASxWSB and CASxCZE expressed this trait in six and eight individuals, respectively. In the B1s, six, two and five individuals expressed this trait in the (CASxWSB)xCAS, (CASxCZE)xCZE and (WSBxCZE)xWSB strains, respectively. This trait is significantly different ($p<0.05$) between parents and hybrids, although this feature isn't equally distributed between crosses and strains (Table 8.4).

The parietal bone showed extra-sutural anomalies in two WSB individuals, and one (CASxCZE)xCZE individual. In the two WSB individuals, these extra sutures appear on the parietal bone. In the backcrossed individual, the right parietal bone exhibited numerous extra sutures and wormion bones (Figure 8.2). This does not appear to be the result of fracture, since the frontal and occipital bone appears to have developed asymmetrically around the parietal bone.

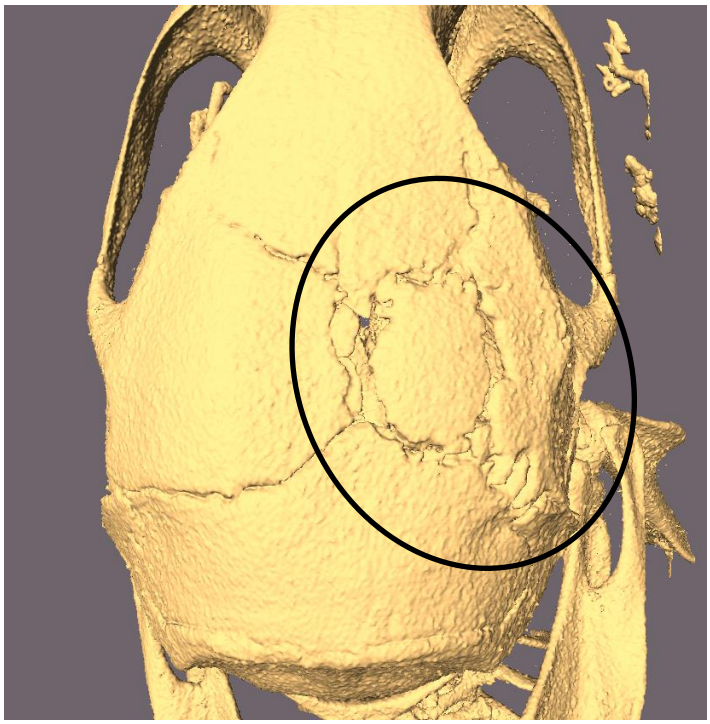


FIGURE 8.2. (CASXCZE)XCZE19 (BACKCROSS) WITH EXTENSIVE SUTURAL ANOMALIES ON RIGHT PARIETAL.

One WSBxCZE F1 individual expressed an extra suture along the right side of the interparietal bone (Figure 8.3). This appears to be associated with a splitting of the mid-cranium, crossing both parietal

bones. This too may be congenital or the result of extensive fracturing, possibly early in life due to the anomalous extra-sutural formation.

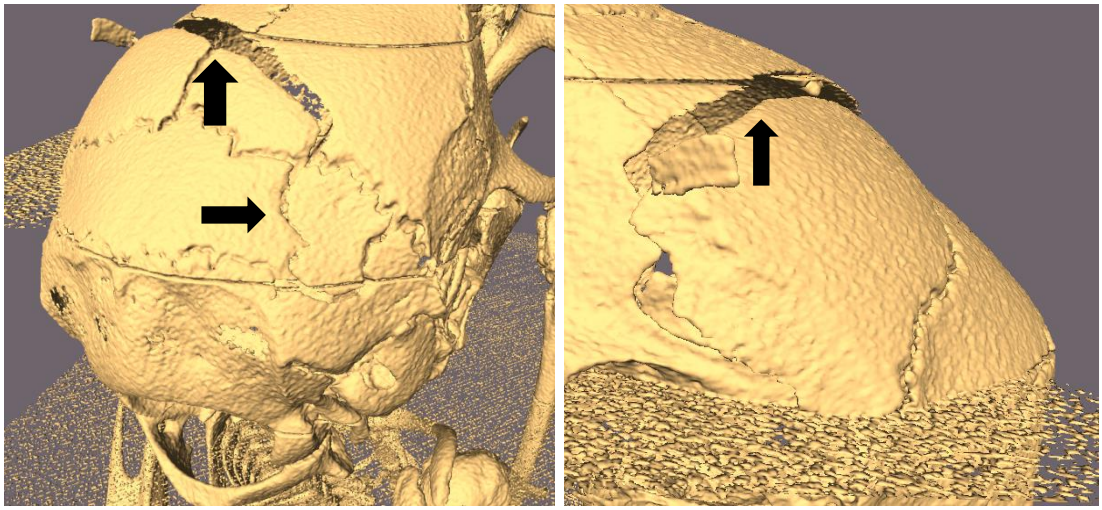


FIGURE 8.3. WSBXCZE19 (F1) WITH UNUSUAL INTERPARIETAL SUTURE AND CROSS-PARIETAL SUTURES OR FRACTURING.

Extra occipital sutures are common in CAS (47 individuals). These sutures are often visible on the left or right side, lateral to the interparietal, and have not been observed on the rest of the occipital bone (Figure 8.4). Only two SPRET individuals exhibit this trait. Among the hybrids, the appearance of this trait is only in CAS recombinants: one CASxCZE F1, eight CASxWSB F2, three CASxCZE F2, 22 (CASxWSB)xCAS. This strongly suggests that this trait is hereditary, but most likely recessive, considering that this trait is more frequent in the (CASxWSB)xCAS backcross, where this is greatest proportion of CAS ancestry, and least expressed in the F1s. However, it must be noted, that this trait is expressed bilaterally in parents in 75.5% of the recorded incidents (although the two SPRETUS individuals exhibited this trait on the right side of the occipital bone only; for CAS alone, the proportion is 79%). In all the hybrids, only 48% of the recorded extra sutures were scored bilaterally. Of all the traits expressed unilaterally, 62.5% (20 out of 32) traits were recorded only on the right side.

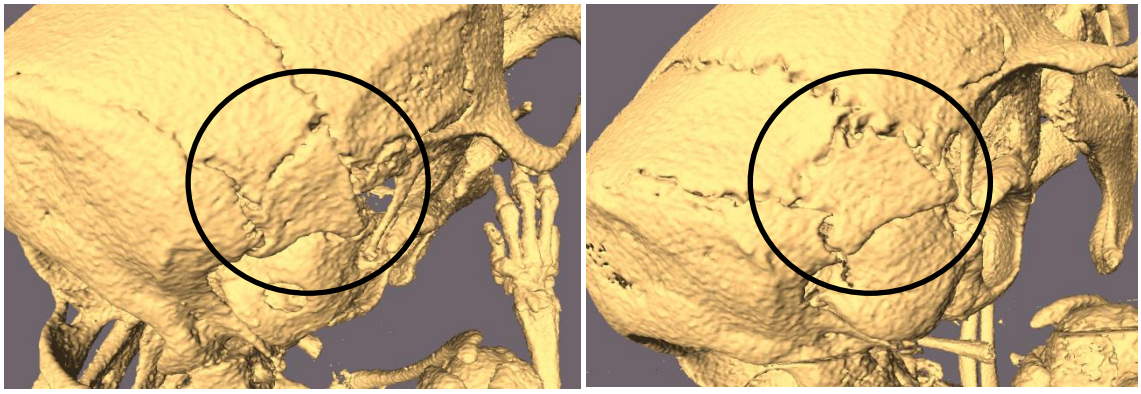


FIGURE 8.4. OCCIPITAL BONE WITH EXTRA SUTURE OF CAST 25 (LEFT) AND CASXWSB13 (RIGHT).

OSSICLES/WORMION BONES

There were no wormion bones visible along the nasal sutures. One (CASxCZE)xCZE individual expressed a bregmatic ossicle and coronal ossicle (due to the extensive sutural anomalies listed above), one (CASxWSB)xCAS individual expressed a lambdoidal ossicle (although this feature is incredibly small; Figure 8.5).

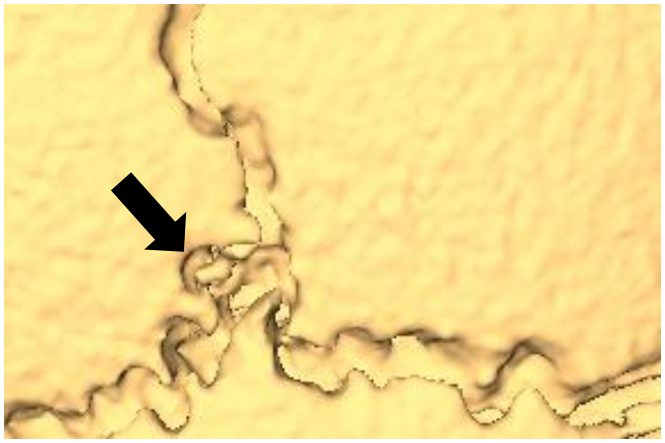


FIGURE 8.5. (CASXWSB)XCAS30 (BACKCROSS) WITH LAMBDOIDAL OSSICLE.

TABLE 8.5. OSSICLES PREVALENT NEAR PROMINENT CRANIAL LANDMARKS.

Strain	n	Lamdoidal	Asterion	coronal	Bregmatic	Pterion	Parietal notch
CAS	50	0	0	0	0	0	0
WSB	50	0	0.48	0	0	0	0.02
CZE	50	0	0	0	0	0	0
SPR	50	0	0.54	0	0	0	0
CASxWSB	50	0	0.02	0	0	0	0
CASxCZE	50	0	0.02	0	0	0	0
WSBxCZE	50	0	0.02	0	0	0	0
SPRxWSB	36	0	0.14	0	0	0	0.06
CASxWSB_F2	50	0	0.04	0	0	0	0
CASxCZE_F2	50	0	0	0	0	0.06	0
(CASxWSB)xCAS	50	0.02	0.02	0	0	0.1	0.02
(CASxCZE)xCZE	48	0	0	0.021	0.021	0	0.042
(WSBxCZE)xWSB	50	0	0.32	0	0	0	0.04
Parental	200	0	0.255	0	0	0	0.005
Hybrid	434	0.002	0.06	0.002	0.002	0.018	0.016
X2		0.461558	**47.16281**	0.461558	0.461558	**3.73375**	1.361004

Ossicles near asterion (Figure 8.6; Table 8.5) are significantly more common in parents: 24 WSB individuals and 27 SPRET individuals. This trait is also recorded in one individual in each intraspecific F1 cross, five SPRxWSB F1 individuals, two CASxWSB F2s, one (CASxWSB)xCAS backcross, and 16 (WSBxCZE)xWSB backcrossed individuals. There is one instance of this trait recorded in a hybrid without it being expressed in either of the parents: the one CASxCZE F1 individual. This is not seen in either of the CAS or CZE parents, or in their F2 or backcross samples. This strongly implies hereditary transmission for this trait, considering that WSB and the backcrossed recombinants with greatest WSB ancestry exhibit this trait in high proportions. However, it is worth noting that the SPRxWSB F1 hybrids do not express this trait in as great a frequency as their parents (10% versus 48% and 54% in WSB and SPR respectively). This may be due to different genetically derived mechanisms affecting expression in the two strains which do not interact in a complementary way. Furthermore, this trait appears to be somewhat masked in the recombinants, implying recessive heredity.

In many cases where asterionic ossicles were observed, it occurred unilaterally (59 individuals, or 75.6%). Out of the traits that were scored unilaterally, 37 (62.7%), were on the right side of the cranium. It is also worth noting that out of the 78 individuals scored with asterion ossicles, 51 were parental and 27 were recombinant. Thirty three percent (33%) of parents scored with this trait

expressed this trait bilaterally compared with 7.4% of hybrids with this trait. Thus, parents appear more likely to express this trait bilaterally.

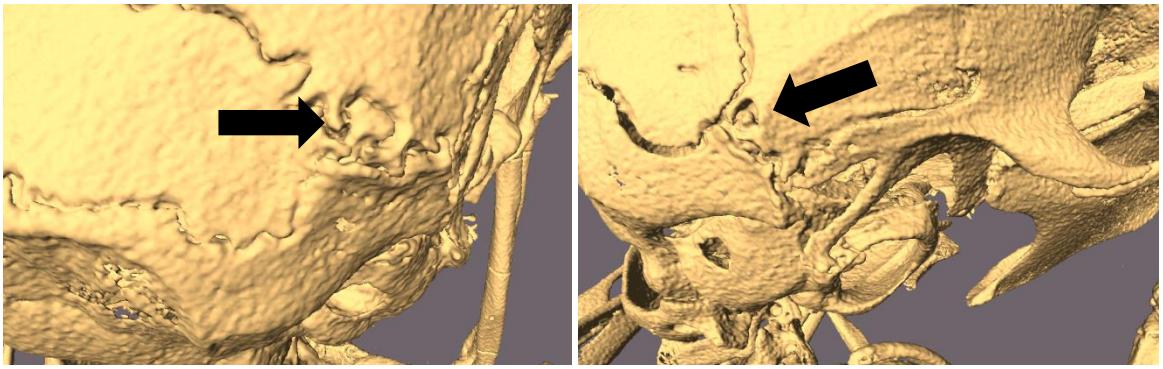


FIGURE 8.6. WORMION BONES NEAR ASTERION FOR WSB13 (LEFT) AND WSBXCZE_CZE 3 (RIGHT).

FORAMINA

The foramina were highly variable among groups, and traits were scored when the number of foramina deviated from what was expected from the literature (Table 8.6). Extra frontal foramina (greater than one frontal foramen) were present in 11.5% of the parents (most common in CAS–11 individuals, and WSB–seven individuals) and 5.3% of the hybrids (most common in CASxCZE F2s–seven individuals, and not present in the interspecific F1 hybrids). Among all individuals with extra frontal foramina, the expression was primarily unilateral (78%).

There was no frontal foramen in 5.5% of parents (most common in CZE–seven individuals and not observed in WSB) and 7.4% of hybrids (in 14 CASxCZE_WSB individuals and eight interspecific SPRxWSB individuals, but none of the WSBxCZE F1s). This trait does not appear to have clear heredity, but is more common in some of the recombinant groups than any in parent group.

Most specimens did not have any maxillary I foramina. They were observed in greatest numbers in the CZE strain (100% of individuals with only 14% displaying this unilaterally). SPRET has no maxillary I foramina in 88% of individuals (but 43% of these are unilaterally expressed). CAS and WSB have this foramen in 44% of individuals each, but in WSB, 71% of these are only expressed unilaterally and in CAS 57% of individuals express this foramen unilaterally. Within the recombinants, 56% of individuals exhibit a lack of maxillary I foramen bilaterally, but 62% are bilateral in the parents. The recombinants are even less likely to have maxillary I: 18% of individuals, especially low in the F2s and B1s.

TABLE 8.6. PROMINENT FORAMINA VARIATION ON CRANIUM AND MANDIBLE.

Strain	n	Frontal		Maxillary foramen I		Maxillary foramen II		Foramen spenoidal ventrale			Postcondylar canal			Preorbital foramen		Mental foramen	Extra sutural incisive foramen
		>1	<1	<1	>1	>1	<1	1	>1	0	<1	>1	>2	<1	>1	>1	>0
CAS	50	0.22	0.06	0.56	0.2	0	0.84	0.36	0.5	0.14	0.06	0.6	0.08	0.54	0.02	0	0.9
WSB	50	0.14	0	0.56	0.04	0.02	0.46	0.4	0.18	0.42	0	1.0	0.48	0.1	0.04	0.94	0.06
CZE	50	0.08	0.14	1.0	0	0.28	0.02	0.36	0.08	0.6	0.02	0.82	0.42	0.48	0.04	0.74	0.6
SPR	50	0.02	0.02	0.88	0.04	0	0.84	0.14	0.06	0.8	0.16	0.6	0.2	0.74	0	0	0.02
CASxWSB	50	0.02	0	0.56	0.08	0	0.64	0.3	0.16	0.54	0	0.9	0.32	0.22	0.02	0.4	0.1
CASxCZE	50	0.04	0.02	0.68	0.08	0.02	0.4	0.46	0.2	0.34	0.08	0.58	0.02	0.02	0.06	0.04	0.9
WSBxCZE	50	0.08	0	0.82	0	0.24	0.08	0.36	0.2	0.44	0.02	0.5	0.36	0.1	0.06	0.92	0.48
SPRxWSB	36	0	0.22	0.92	0	0.03	0.08	0.36	0.5	0.53	0.11	0.75	0.22	0.31	0.14	0	0.11
CASxWSB_F2	50	0.04	0.02	0.9	0	0	0.42	0.4	0.42	0.18	0.08	0.68	0.34	0.3	0.14	0.36	0.18
CASxCZE_F2	50	0.14	0.08	0.96	0.02	0.1	0.16	0.42	0.36	0.22	0.28	0.5	0.1	0.38	0.2	0.14	0.62
(CASxWSB)xCAS	50	0.06	0.04	0.84	0.02	0.02	0.72	0.32	0.72	0	0.06	0.66	0.16	0.24	0.26	0.2	0.36
(CASxCZE)xCZE	48	0.02	0.29	0.92	0	0.23	0.08	0.23	0.125	0.69	0.17	0.54	0.15	0.48	0.15	0.17	0.81
(WSBxCZE)xWSB	50	0.06	0.04	0.78	0	0.3	0.12	0.22	0.52	0.26	0.08	0.58	0.22	0.1	0.36	0.66	0.24
Parental	200	0.115	0.055	0.75	0.07	0.08	0.54	0.31	0.21	0.49	0.06	0.76	0.295	0.31	0.025	0.42	0.395
Hybrid	434	0.053	0.074	0.813	0.023	0.11	0.309	0.341	0.353	0.348	0.097	0.629	0.21	0.099	0.152	0.332	0.431
X2		**7.8**	0.76	**3.6**	**8.29**	1.5	**31.0**	0.8	**14.0**	**11.6**	2.38	**9.8**	**5.5**	**77.1**	**22.2**	**4.6**	0.72

Having more than 1 maxillary I is rarer, but occurs in highest proportions in CAST (20%), and CAST F1s (8% in CASxWSBF1s and CASxCZE F1s). It is also seen in two SPRET, one CASxCZE F2 and one (CASxWSB)xCAS individuals. Some individuals even express two maxillary I foramina on one side and none on the other (one of each: SPRET, CASxWSB F1, CASxCZE F1 and (CASxWSB)xCAS).

Maxillary II foramina are highly variable in terms of expression. Parents are likely to have no foramen: expressed in only 16% of CAS and SPRET individuals and 54% of WSB individuals (although it must be noted that in WSB this is mainly unilateral). Among the recombinants, this trait is least common in CAS recombinants, particularly with WSB (36% CASxWSB F1s, 60% CASxCZE F1s, 58% CASxWSB F2s and 28% (CASxWSB)xCAS B1s) and is far more likely to be unilaterally expressed (86% in recombinants versus 38% of parents). More than one maxillary II foramen is rarer, although in higher proportions in CZE (28%), and some CZE recombinants (WSBxCZE F1–24%, (CASxCZE)xCZE–22% and (WSBxCZE)xWSB–30%). This is expressed bilaterally in 27% of parents and 28% of recombinants.

Multiple preorbital foramina (more than one per side) occur more often in hybrids (15.2%) than in parents (2.5%). It is seen in between zero and two individuals in each of the parent strains, and is highly variable among recombinants, occurring more rarely in the F1 individuals (one CASxWSB, three of each CASxCZE and WSBxCZE, and five SPRxWSB). It is most common in the (CASxWSB)xCAS (26%) and (WSBxCZE)xWSB (36%) backcrosses. However, this was not an easy trait to observe on the scans and interpretations should be made with caution. Despite this, it is more likely that this trait is underscored than overscored, and the expression of this trait in mouse crania warrants further research.

Extra mental foramina were seen in high frequencies in two of the parents (WSB-94%- and CZE-74%), but were not seen in CAS and SPRET. Among the hybrids, this trait was seen in greater frequencies in all CZExWSB recombinants (as high as 92% of the CZExWSB F1s, 66% of the (WSBxCZE)xWSB B1s), but was not seen in the interspecific cross, SPRxWSB. This supports a hereditary nature of this trait, although there are complexities. The (WSBxCZE)xWSB B1s are far smaller than expected for simple Mendelian heredity and there are high levels of this trait seen in CASxWSB recombinants (F1s–40% and F2s–36%), but not in CASxCZE individuals (F1s–4% and F2s–14%). This may imply different mechanisms of heredity for the trait from both parents.

Similarly, extra sutural incisive foramina are more common in two of the parents (CAS–90%, and CZE–60%) and in their recombinants (CASxCZE F1s–90%, F2s–62%, and (CASxCZE)xCZE–81%). This feature is lowest in SPRET (one individual) and the inter-specific hybrid (8%; four individuals). It is worth noting that when this feature is expressed, it is bilateral in 65% of the cases, but is highly variable per strain.

It is bilateral at higher frequencies in CZE, CASxWSB F1s, CASxCZE F1s and (CASxCZE)xCZE B1s (also in SPRxWSB; but there are only four individuals). This appears to be more commonly bilateral in F1s, but WSBxCZE F1s are bilateral in only 37.5% of the times they are scored with the trait.

HYPERSTOTIC/HYPOSTOTIC TRAITS

Bridging on incisive foramen has been observed in only four individuals: two CASxCZE F2s, one CASxWSB F2 and one (CASxWSB)xCAS B1 (Table 8.7). A frontal fontanelle was only scored in one CASxWSB F1 and one (CASxWSB)xCAS B1.

Parted frontal bones appear significantly more frequently in recombinants, with only two CZE specimens expressing the trait (1% of all parents) compared with 8.3% of hybrids. Among hybrids, this trait is seen in 14 CASxWSB F1s, five WSBxCZE F1s, eight (CASxWSB)xCAS B1s, four (WSBxCZE)xWSB B1s and two CASxWSB F2s. A single individual from each of the following groups also exhibits this trait: CASxCZE F1s, CASxCZE F2s and (CASxCZE)xCZE B1s. The interspecific hybrids (SPRxWSB) do not appear to exhibit this trait.

TABLE 8.7. HYPERSTOTIC/HYPOSTOTIC TRAIT VARIATION ON MICE CRANIA.

Strain	n	Bridging on incisive foramen	Parted frontal bones	Frontal fontanelle
CAS	50	0	0	0
WSB	50	0	0	0
CZE	50	0	0.04	0
SPR	50	0	0	0
CASxWSB	50	0	0.28	0.02
CASxCZE	50	0	0.02	0
WSBxCZE	50	0	0.1	0
SPRxWSB	36	0	0	0
CASxWSB_F2	50	0.02	0.04	0
CASxCZE_F2	50	0.04	0.02	0
(CASxWSB)xCAS	50	0.02	0.16	0.02
(CASxCZE)xCZE	48	0	0.02	0
(WSBxCZE)xWSB	50	0	0.08	0
Parental	200	0	0.01	0
Hybrid	434	0.009	0.08	0.005
X2		1.855	**12.93**	0.92

OBSERVED TRAITS

SUTURAL COMPLEXITY

Sutural complexity is highly variable among strains and crosses (Table 8.8). SPRETUS has the greatest proportion of individuals with highly convoluted parietal sutures with 92% of the strain being scored with 3 or 4. WSB and CZE also display highly convoluted parietal sutures (64% and 30% respectively). CAS has more individuals with straighter sutures (only 12% scored 3 or 4). Among hybrids, recombinants of WSB and CZE have the greatest proportions of highly convoluted sutures (60% of F1s, 34% of Backcrosses with WSB, and 34% of F2s). Recombinants of CAS have lower proportions of convolution. This implies some heredity of parietal convolution.

TABLE 8.8. PARIETAL SUTURE COMPLEXITY.

Strain	n	5	3 and 4
CAS	50	0.1	0.12
WSB	50	0.14	0.64
CZE	50	0	0.3
SPR	50	0	0.92
CASxWSB	50	0.08	0.6
CASxCZE	50	0	0.18
WSBxCZE	50	0.22	0.36
SPRxWSB	36	0	0.472
CASxWSB_F2	50	0.1	0.34
CASxCZE_F2	50	0.04	0.26
(CASxWSB)xCAS	50	0.04	0.18
(CASxCZE)xCZE	48	0	0.104
(WSBxCZE)xWSB	50	0.08	0.68
Parental	200	0.06	0.495
Hybrid	434	0.065	0.35
X2		0.047	**11.997**

The SPRxWSB F1 hybrid also has a high proportion of convolution (34%) but is far lower than either SPRET or WSB proportions. This may be because the underlying genetic causes of convolution are not the same for the two species, and therefore are non-complementary. It is worth noting that WSB and SPRET have different convolution morphologies, with SPRET having a more "wavy" convolution and WSB have a more erratic convolution.

An additional feature that was scored was for sudden deviations in an otherwise straight parietal suture. These deviations (scored "5") are also recorded as to whether they are deviating to the left or right, and whether they occur near Lambda, Bregma or the middle of the suture. This feature in parents is at high frequencies for WSB (14%) and CAS (10%), and does not appear in CZE or SPRET. It presents in mainly WSB intraspecific recombinants, with highest proportions present in WSBxCZE (22%), although it is also seen in 2 CASxCZE F2 individuals. It is not present in CASxCZE F1, SPRxWSB F1 and (CASxCZE)xCZE individuals. These deviations are more likely to be near Lambda (65% of these features, with 22.5% near bregma and 12.5% in the middle), and are more likely to deviate to the right (67.5%).

OCCIPITAL/ATLAS DEFORMITY

Within the strain SPRET, many individuals had clearly deformed occipital regions, with fusion of the atlas to the occipital condyles, posterior opening of the atlas, and/or duplication of the occipital condyles (Figure 8.7.). These deformities were often also associated with a displacement within the basicranial region, which could be noticed throughout the lower parts of the cranium, ultimately placing the mandible in a more anterior position than in other strains. This "under-bite" likely contributes to the high level of dental wear and (ultimately) mandibular M3 loss seen in this strain. However, this is not seen in the WSBxSPR hybrids. This is possibly due to the inbreeding effect of SPRET and deformities in the founder parents of this strain, though it may be present naturally in this species as well.

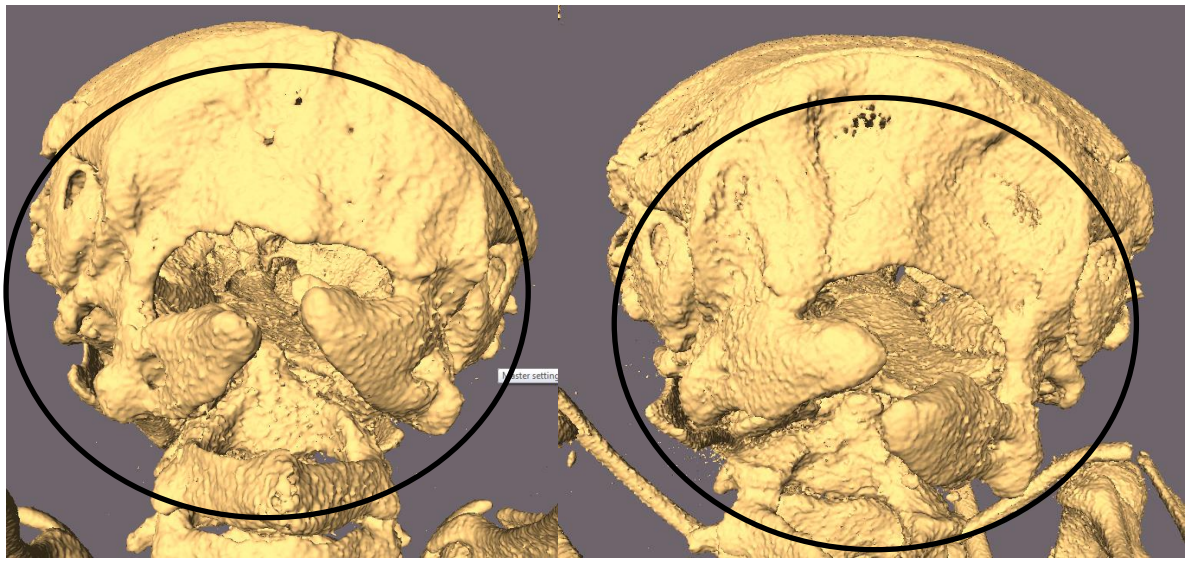


FIGURE 8.7. OCCIPITAL DEFORMITY IN SPRETUS STRAIN (7 AND 24).

"Ayla was right. He's not deformed, he's a mixture, a mixture of her and Clan... Creb shook his head, he didn't know, but it set the old magician to thinking. He thought often of Durc that cold lonely winter. He had a feeling Durc was important, but just why eluded him."

Clan of the Cave Bear, Jean M. Auel

CHAPTER 9

DISCUSSION AND CONCLUSION

The main aim of this thesis was to characterise the cranio-mandibular morphology of mouse hybrids, in order to create an animal model for hybrid skeletal morphologies. This will, ultimately, be extrapolated onto the fossil record. These data provide an experimentally controlled, multigenerational model to substantially expand the (somewhat limited) information we currently have on mammalian hybrid skeletal morphologies. In this chapter, the results from the three preceding chapters are summarised. They are then discussed within the context of the literature on mammalian hybrid morphologies, and on mouse (*Mus musculus*) hybridization specifically. It is then possible to evaluate each of the hypotheses laid out in Chapter 5 (Materials and Methods). These results are used to establish a baseline model for understanding how hybridization affects the cranium given varying degrees of phylogenetic distance among parents. The collated results are then further evaluated within the context of development, specifically how our understanding of skeletal development may explain some of the patterns present in the morphology of hybrids. Finally, these patterns are compared with individual specimens and samples within the hominin fossil record. The

chapter concludes with a summary of the limitations of this research; suggestions for further research, stimulated by the results of this thesis and the hybrid mouse project, as a whole, will also be made.

Summary of Results

Cranio-mandibular form of the first generation mouse hybrids: A Summary of chapter 6

This chapter is a manuscript, currently in review, intended to bridge the "gap" between research conducted on baboon, gorilla and tamarin hybrids (Ackermann, Rogers *et al*. 2006, Ackermann, Bishop 2010, Cheverud, Jacobs *et al*. 1993), and the larger Mouse Hybrid Project (PI. Rebecca Ackermann). The methodologies are therefore similar to those used in the above papers. A large proportion of this manuscript is relevant to the purpose of this thesis. Considering the author's contributions to the paper lay primarily in the cranio-mandibular analyses, postcranial results will not be discussed (although may be viewed within the manuscript).

As demonstrated in Chapter 6, all three intraspecific F1 hybrids display overall transgression in cranial and mandibular size, with hybrids significantly larger, in terms of their geometric means, than either of their parents, apart from the CASTxWSB F1 mandible as compared with the largest parent strain (WSB; in this case, they were comparable in size to one another). Therefore, craniomandibular size is larger than an average parental value in all intraspecific crosses, and frequently outside the range of both parents. Cranial and mandibular shape, however, appeared intermediate in F1 hybrids relative to parents, but was transgressive in some measures (most clearly for PC2 of the PCA). These measures included cranial height (shorter in hybrids) and snout length (longer in hybrids).

All six of the groups that were compared differed significantly in measures of form, which were highlighted within the analysis. Parents differed from each other in measures of facial length, temporal width, mandibular alveolar length and coronoid height. Hybrids differed from each other in frontal bone length, occipital length and mandibular antero-posterior measures. However, interesting for the purposes of this thesis, there were measures consistently larger in all three hybrid crosses compared with their parents: occipital length and breadth, temporal width ("flare") and mandibular alveolar measures. Most measures were larger than the midpoint values in F1 hybrids, often overlapping greatly with the larger parent. Fifteen measures (of 38) were transgressive (larger than both parents) in all three hybrids compared with parents. These measures represent palatal and facial length, parietal width and cranial height, as well as mandibular alveolar lengths.

Multigenerational recombinants and the Effects of Differences in Phylogenetic Distance on hybrid morphology: A Summary of chapter 7

Within Chapter 7 the results were separated out into five sub-sections in order to create cohesion among related results. Parents, F1 hybrids, F2 hybrids and one backcross were analysed for each of the three intra-specific groups. In terms of cranial and mandibular size, F1 generations were the most extreme (largest) of all crosses. Subsequent generations displayed intermediate cranio-mandibular size between the parents and the F1 hybrids, and exhibited larger (albeit not significantly so) size variation. However, size was not always a useful variable for classification within groups. While mandibular size increased with greater proportions of F1 hybrids, and variance fluctuated in a negative parabolic pattern, these predictions were often non-significant at proportions expected in a natural population (e.g. individuals of F1 size form less than 50% of the sample). For cranial size, however, the inclusion of hybrids within a mixed sample greatly increased the mean cranial size and variance of the sample and the trend was far more compelling. This implies that overall cranial size may be used to indicate whether hybridization has occurred within a sample.

In terms of shape, the patterns seen in mandibles and crania are consistent with expectations based on quantitative genetic theory. The F1 hybrids are intermediate to parents, the B1s are intermediate to the F1 hybrids and the parents with which they are backcrossed, and the F2s overlap with the F1s, yet show greater morphological variance. In the cranium, this pattern is particularly clear when size-related shape is regressed out, although there is far more overlap among groups. B1s and F2s are more variable in shape than either of the parents or F1s. This is particularly marked in the mandible. Furthermore, shape data appears to be more powerful than size to determine classification within strains. These data have shown that F2s more closely resemble F1s in cranial and mandibular shape, although some individuals exhibit parental features. The B1s however exhibit shape that closely resembles both F1s and the backcrossed parent.

In the WSB/SPR (inter-specific) hybrid, the patterns differ somewhat from what is seen in the intra-specific hybrids. For instance, while cranial size is larger than the parental midpoint value, it is smaller than the larger parent (and therefore not transgressive like in the intraspecific crosses). Size and size-related shape largely reflect differences between parents (SPRET being larger on average), and appear to reflect changes in cranial height. However, when looking at shape variables, the F1 hybrid is intermediate in much of the morphology, and the greatest shape differences are seen between the parents. However, once again, there are some aspects of shape morphology which appear outside of the variation of the parents. Hybrids, for instance, appear to exhibit wider, more anteriorly protruding faces, with longer maxillary measures.

When the cranium is broken up into two modules of the face and cranium, the only strains without significant modularity in the hypothesized elements are SPRET and the inter-specific hybrid (WSBxSPR), although it is more modular in the parent. Similarly, the hypothesized modules of the face/basicranium/neurocranium are significantly modular (relative to other random partitions of the cranium) in CAST and its recombinants, CASxWSB F1 and CASxWSB F2, but not in other strains. The only two strains that showed significant modularity in the anterior and posterior modules were two parent strains (CZEand WSB).

Covariance correlations between strains were calculated for two modules of both the cranium and mandible. In the mandible, we can see general patterns of correlation in the different groups. For instance: covariance matrices of modules of F1 hybrids were often significantly (and tightly) correlated with those of parents; and F2 hybrids were tightly correlated with F1s and parents. B1s were highly variable in covariance correlations. It is worth noting that, in some instances, F1s or F2s were more tightly correlated with one parent than with the other in the anterior mandibular module, and vice versa in the posterior mandibular module. This is fairly striking in CASxCZE F1 and F2 hybrids compared with parents, CASxWSB F1 to parents, and CZExWSB F1 compared with parent. Backcrosses are also difficult to deduce patterns for in the mandibular modules where covariance correlations are not predictive of the backcrossed parent, nor necessarily like that of the F1 hybrid.

In the cranium, similar discordance between F1s and parent correlations is seen in the facial and neurocranium modules: in the CASxCZE F2 compared with parents; and in the CZExWSB F1 to parents. However, all covariance matrices were significantly correlated among strains in the cranium. In CASxCZE F1s, CASxWSB F1s and F2s, and SPRxWSB F1s, however, covariance was more strongly correlated with the same parent for both modules (CZE, WSB and WSB, respectively). In the cranium modules, the covariance matrices of the B1s appear to more tightly correlate with the parents with which they are backcrossed.

Non-Metric Trait Variation in Hybrids and Recombinants: A summary of chapter 8

Non-metric traits on the cranium, mandible and dentition have been suggested to be indicators of a breakdown of coordination in development within strongly integrated systems (Ackermann *et al.* 2006, Ackermann 2007). Within this chapter, some dental anomalies were rarely, or never, recorded, such as supernumerary teeth and tooth rotation. Some dental traits were probably indicative of lifestyle and stress levels, rather than being inherited traits. Missing, broken and worn teeth were significantly more common in parent strains than hybrids and recombinants. This is likely owing to

wild-derived parents, in particular SPRET, being more stressed in captive environments (de Freitas and Humphreys, pers. comm), and the hybrids displaying a form of "hybrid vigour" by remaining relatively calm in captivity (seen in domesticates and hybrids, such as mules).

There are a few traits which are more common in hybrids than in parents. Intra-specific hybrids and recombinants were more likely to exhibit more than 4 cusps on M2s. The inter-specific hybrids were more likely to exhibit extra zygomatic sutures (44%), although this trait was also observed in some of the post-F1 intra-specific crosses (between 4% and 16%), and some CAS and SPR individuals (12% and 4% respectively). CAS/WSB hybrids and recombinants were also more likely to exhibit parted frontal bones (28% in F1s, 16% in B1s). Furthermore, only one CASxWSB individual and one (CASxWSB)xCAS individual exhibited a parted frontal fontanelle. Hybrids were also more likely to not exhibit maxillary foramen I, to have more than one foramen sphenoidale ventral and to have more than one preorbital foramen (although the latter is difficult to observe with certainty on scans).

It is also important to note striking individuals. In one backcrossed individual ((CASxCZE)xCZE), numerous wormian bones were observed on the right parietal bone. The intricate number of wormian bones affected the laterality of the bone relative to the left parietal bone and thus appeared to affect the symmetry of the interparietal and frontal bone. Another individual, a WSBxCZE F1, appeared to have a highly unusual suture running across both parietal bones and another antero-posteriorly along the right side of the interparietal. One interspecific (WSBxSPR F1) individual exhibited what appeared to be a right maxillary suture. It is uncertain as to whether traits result from hybridization or some extrinsic factor (e.g. mechanical stress), but it is likely they all occurred during development, since the bones surrounding the sutures appear to have formed normally around them.

SPRET was a particularly unusual parental cross. This is particularly noticeable in the atypical morphology of the occipital condyle/atlas region. It is possible that this morphology is the result of inbreeding, though without samples from the wild this is difficult to know. If it is an inbreeding effect, the lack of these features in the hybrids might be the result of outbreeding of the strain. It appears that the atypical occipital condyle/atlas morphology may have even affected the relative position of the mandible, increasing the likelihood of dental wear or loss. SPRET was also the only strain in which nasal fusion was present, and had high proportions (54%) of individuals with ossicles at asterion.

Other parents displaying some non-metric anomalies include WSB, which were more likely to exhibit basisphenoid-presphenoid fusion as well as asterion ossicles; CAS, which was more likely to be scored for extra occipital sutures and no preorbital foramen (although this trait is not easy to observe on scans); WSB and CZE, which have high proportions of individuals with more than one mental foramen

(94% and 74%, respectively); and CAS and CZE with high proportions of extra sutural incisive foramina (90% and 60%).

Some of these traits appear to be inherited in subsequent generations. Occipital sutures are most common in CAS (94%) and were also observed in 44% of the (CASxWSB)xCAS backcrosses. It is likely that this trait is not dominant, considering its relative rarity in F1s (one CASxMUS individual) and F2s (11%). Similarly, among recombinants, asterion ossicles were most frequent in (WSBxCZE)xWSB (32%), implying that this trait is inherited. However, the pattern of heredity is not easy to ascertain, given that only 10% of SPRxWSB F1 hybrids exhibited this trait, despite both parents exhibiting high proportions. Similarly, very few of the other WSB recombinants have asterion ossicles (one or two per group), and one CASxCZE F1 was scored for exhibiting an asterion ossicle despite neither parent exhibiting this trait.

Parietal sutural complexity was observed to be highly variable among strains and crosses. High levels of very convoluted sutures are observed in the parents, SPR (92%), WSB (64%) and CZE (30%). Furthermore, high levels of sutural complexity were also observed in WSB recombinants (60% of CASxWSB F1s and 68% of (WSBxCZE)xWSB), and comparatively low levels in CAS/CZE recombinants. However, the pattern of heredity is not clear, and, despite being scored for high levels of sutural complexity in both SPR and WSB, it is observed that the patterns of convolution are highly variable among strains and individuals. Marked sutural complexity may be more "wave-like" (such as noted in SPRETUS) or may be erratic in different individuals (WSB and recombinants).

ASSESSMENT WITHIN THE FRAMEWORK OF EXISTING LITERATURE

CRANIAL FORM AND HETEROSIS IN PREVIOUS HYBRID STUDIES

Several studies have compared size and shape in the skulls of hybrids and their parent taxa. For instance, despite having diverged relatively recently (350ka BP; Zinner, Wertheimer *et al*. 2013), hybrids between olive and yellow baboons were shown to exhibit heterosis (larger than midpoint of parents) in certain measurements. The F1 hybrids were significantly larger than both parents in only one of these measures, occipital length (Ackermann, Rogers *et al*. 2006). The F1 hybrids were larger-than-parental-midpoint in 72% of traits, and significantly so (p=0.05) in 8% (Ackermann, Rogers *et al*. 2006). Backcrossed hybrids were also analysed in this study. Here they were greater than parental midpoint in 49% of traits (significant in 18%: occipital length, facial height and cranial length), and

significantly transgressive in cranial height. Larger-than-parental-midpoint measures were also found within the nasal cavity of these hybrid baboons (Eichel 2014, Eichel, Ackermann 2016).

In saddle-back tamarin hybrids, F1 crosses between *Saguinus fuscicollis illigeri* and *S. f. lagonotus* were significantly larger in 2 of the 39 measurements compared with parents (around the frontal alveolar region and around the occipital), and larger than parental average in all traits (56% significantly so; Cheverud, Jacobs *et al*. 1993). In the *S.f. illigeri* and *S. f. leucogenys* cross, there are comparably fewer significant differences among parents and F1 hybrid. However, four of the 39 measurements were significantly larger than the expected parental midpoint, and is larger (although not necessarily significantly so) in 67% of measures. In both crosses, tamarin hybrids are shown to be significantly larger than one parent, and significantly larger than expected (parental midpoint; Cheverud, Jacobs *et al*. 1993). While *S. f. leucogenys* has since been shown to have genetic incongruity, hinting at historic hybridization among subspecies (and possibly explaining the lower heterosis in these hybrids), the *Saguinus fuscicollis* clade in general appears to have diverged around 3 Ma (Matauschek, Roos *et al*. 2011).

In both the baboons and tamarins, external cranial heterosis is far more subtle than that seen in the intra-specific mouse hybrids. This could be due to several non-mutually-exclusive factors: variability in accumulated mutations which effect different hybrids in different ways, inbreeding depression within the mouse parents, and patterns of size heterosis which may be influenced by uncoordinated epigenetic effects governed by phylogenetic distances between parent taxa. The latter scenario will be more thoroughly considered later in the chapter.

Another important feature is that for all three taxa (mice, tamarins and baboons), measures in the occipital bone were significantly larger than midparental average or even larger than the parents. Furthermore, measures related to tooth row length, in both the mandible and maxilla, are also highlighted as larger than expected in all three studies. This implies that, regardless of degree of heterosis or transgression of cranial and mandibular elements as a whole, the occipital region and mandibular/ maxillary alveolar regions of the skull are sensitive to the additive effects of hybridization.

Among gorillas, heterosis in *Gorilla beringei graueri* compared with *G. b. beringei* and *G. g. gorilla*, has been proposed as supporting hybridization until about 80 Ka, with initial divergence around 400 Ka (Ackermann, Bishop 2010). The implication is that hybrid morphologies are retained in the hybrid *G. b. graueri* population. In this scenario, hybrid morphologies may be retained long after the introgression events have taken place. However, genetic drift, rather than ancient hybridization, may

be the more parsimonious explanation for unusual morphologies in *G. b. graueri* (Tocheri, Dommain *et al*. 2016).

M. M. MUSCULUS/*M. M. DOMESTICUS* HYBRIDS: ESTABLISHING PATTERNS

Much of the research on mouse hybridization focusses on the mouse hybrid zone between *M. m. musculus* and *M. m. domesticus* (in this thesis, represented by CZE and WSB, respectively). The importance of this research, however, lies in the fact that both inbred strains (such as those used here) and recombinants from within the natural hybrid zone have been studied. This allows for some comparisons among what is studied in the laboratory and the usefulness of this research in its application to natural hybridization. Furthermore, the comparisons among these hybrids, and the other crosses (inter- and intra-specific hybrids) studied within this thesis, also provide support for consistency of certain hybrid morphologies among taxa.

Consistent with the trends noted in this thesis, mandibular and cranial transgressive size has been detected in studies of inbred mice representing *M. m. musculus/ M. m. domesticus* subspecies, and among different laboratory mice (Renaud, Alibert *et al*. 2009, Renaud, Alibert *et al*. 2012, Chai 1956, Percival, Liberton *et al*. 2016a, Thorpe, Leamy 1983, Leamy, Thorpe 1984, Leamy 1982). Furthermore, such studies also show intermediate mandibular shape when size is removed (Renaud, Alibert *et al*. 2012, Renaud, Alibert *et al*. 2009). However, contrary to what is seen in this analysis, shape variance of the mandible was shown to be far larger in F1 hybrids than parents (Renaud, Alibert *et al*. 2009). Here we see an increased variance in recombinants, but not necessarily in F1s.

In the *M. m. musculus*/*M. m. domesticus* natural hybrid zone, however, this pattern is not as clear. Shape change across the hybrid zone is continuous: from a more *M. m. musculus* craniofacial shape in the eastern part of the hybrid zone, to a more *M. m. domesticus* shape in the western parts of the hybrid zone (Pallares Amaya 2015, Pallares, Turner *et al*. 2016a, Auffray, Alibert *et al*. 1996a). This has important implications for the underlying genetic architecture governing cranio-mandibular morphology (i.e. supporting the polygenic underpinnings of craniofacial shape; see Klingenberg, Navarro 2012). However, these studies show a weak correlation between allelic proportions contributed by the mice and cranio-mandibular size, and size does not increase towards the centre of the hybrid zone (Pallares, Turner *et al*. 2016a). This implies that overall shape morphologies should be proportional to genetic contributions from parents: which is supported by research within this thesis among all groups studied.

Some studies have looked at fluctuating asymmetry of cranial, mandibular and dental measures in hybrid mice, as a proxy for assessing developmental stability (Alibert, Fel-Clair *et al*. 1997, Auffray, Alibert *et al*. 1996b, Alibert, Renaud *et al*. 1994, Alibert, Auffray 2003). These studies show that, despite incompatibilities in the parents which affect hybrid fertility, hybrids had lower molar and mandibular fluctuating asymmetry than parents (Alibert, Renaud *et al*. 1994, Renaud, Alibert *et al*. 2009). This was lower in laboratory hybrids, but the pattern was still observed in the wild (Alibert, Fel-Clair *et al*. 1997, Auffray, Alibert *et al*. 1996b, Alibert, Renaud *et al*. 1994). This pattern, however, was not observed on the ventral side of the cranium in the European hybrid zone (Mikula, Macholán 2008). Developmental stability in hybrids relative to parents, due to increased heterozygosity, may be seen here in the relative stability of the occipital condyle morphology in the inter-specific hybrids relative to one of the parents, SPRET.

Patterns of integration explaining hybrid variation

While hybridization is often seen as a force which reduces variation between groups (i.e. makes groups more similar), the effects are highly variable (see chapter 2). One such effect, corroborated in this study, is that recombinants are highly variable in size and shape. Variation in hybrids has previously been seen as a potential source of novelty in populations (Renaud, Alibert *et al*. 2012, Parsons, Son *et al*. 2011, Valentin, Sévigny *et al*. 2002, Stelkens, Schmid *et al*. 2009, Selz, Lucek *et al*. 2014). This has been attributed to modularity; changes to the patterns/degree of integration in mandibular modules has been suggested as one reason for the emergence of novel phenotypes in mouse hybrids (Renaud, Alibert *et al*. 2012). Since the crania and mandibles of hybrids within this study have been shown to be extreme in both size and (partially) in shape, with high degrees of variability in recombinants, this may explain the patterns seen here. Another option, demonstrated in cichlid hybrids, is a relaxation of constraints on covariation, and therefore a change in the magnitude of integration (Selz, Lucek *et al*. 2014, Stelkens, Schmid *et al*. 2009). Such relaxation of constraints may also explain novel external morphologies, such as mosaic pelage patterning in howler hybrids, atypical orofacial pelage variation and variation in body measurements in marmoset hybrids (Aguiar, Mellek *et al*. 2007, Aguiar, Pie *et al*. 2008, Fuzessy, de Oliveira Silva *et al*. 2014).

Modularity of the face and cranium was supported in most strains analysed here, except for SPRET and the interspecific hybrid, but not necessarily for the facial/basicranium/neurocranium modules proposed or for the anterior/posterior mandible. Covariance matrices of the anterior and posterior mandible were significantly correlated between F1s and parents and between F2s and F1s. However, it is worth noting that correlations were not necessarily similar between F1s and both parents or F2s

and both parents. For instance, in the CAS/CZE group, F2s were correlated with CAS in the anterior mandible at 0.82 (p=0.0002) and with CZE at 0.65 (p=0.005). The posterior mandible indicated no such discrepancy (0.64 to 0.63, respectively). Comparable discrepancies between F1s and the two parents, or B1s and its parents (F1s and parent strains), were seen in all comparisons in the mandible. Similarly, differences in correlations between F1s, F2s and parents were seen in the cranium, both in magnitude and direction (much higher correlation with one parent in the face, higher correlation with the other parent in the cranium). These discrepancies support a scenario of differential integration of modules of the cranium being a potential source of variability and novelty in hybrids.

It is worth noting that in multigenerational recombinants (particularly B1s) covariance matrices of the mandibular modules were often not significantly correlated with parents, F1 hybrids or even each other. These correlations were also among the smaller compared with each of the other strains hinting at dis-integration in the mandible in these generations. This pattern is not seen in the cranium. What is noticeable in the cranium is that covariance correlations are smaller (although still significant) among inter-specific hybrids and their parents, versus intra-specific hybrids and their parents.

Detecting Hybridization using non-metric traits

Ackermann and colleagues have noted a higher proportion of atypical non-metric traits (absent or rare in parent populations) in hybrids (Ackermann, Schroeder *et al*. 2014, Ackermann, Brink *et al*. 2010, Ackermann, Rogers *et al*. 2006). In studies looking at baboons, samples of hybrids and recombinants were highly variable in size. However, significant differences were recorded among: (1) male hybrids for supernumerary teeth, (2) female hybrids for dental crowding, and (3) both males and females for atypical sutures and remnant metopic sutures (Ackermann, Schroeder *et al*. 2014, Ackermann, Rogers *et al*. 2006). The majority of the supernumerary teeth scored were distomolars, although one individual had full-sized bilateral supernumerary maxillary canines, an anomaly only recorded once in the literature in a baboon from a natural hybrid zone (Ackermann, Rogers *et al*. 2006). Distomolars were largely seen in F1 males (50% of 18 individuals recorded in the 2006 paper, over 30% of 29 individuals in the later study), and were often bilateral, mandibular and full-sized. This was compared with supernumerary teeth in the parents, which were extremely rare, and typically reduced in size, maxillary and unilaterally expressed (Ackermann, Rogers *et al*. 2006). In addition, a female hybrid with severely reduced canines, and two individuals with rotated teeth were observed in the hybrid samples (Ackermann, Schroeder *et al*. 2014). In this later study, unusual sutural features, such as extra zygomaxillary sutures, ossicles and spicules, were shown to be more prevalent in hybrids compared with parents (Ackermann, Schroeder *et al*. 2014). Ackermann and colleagues (2006) pointed out that,

in a hybrid sample with relatively low levels of heterosis, unusual non-metric traits were a clearer indication of hybridization, and therefore more useful for interpreting the fossil record.

In other mammals, a suite of otherwise rare morphological traits have been recorded in recombinants. Supernumerary teeth in recent and late Pleistocene ground squirrel populations in hybrid zones have also been noted. In this case, the squirrels exhibited bilateral maxillary supernumerary dentition (Goodwin 1998). Similarly, dental abnormalities present in a beluga/narwhal hybrid cranium have been observed (Heide-Jørgensen, Reeves 1993). Hybrid wildebeest (between black- *Connochaetes gnou*- and blue wildebeest- *C. taurinus*, which diverged approximately 1 Ma) also display suite of dental, sutural and horn anomalies (Ackermann, Brink *et al*. 2010). Among 13 wildebeest hybrids, one had a rotated premolar and six had sutural anomalies, which were not observed in parent taxa.

Considering dental anomalies appear common within this literature, it may seem strange that supernumerary dentition and tooth rotation do not feature within the mouse data. This may be due to several reasons. Firstly, development of mouse dentition differs between mice and primates. Mice have incisors which are continually growing, and a reduced number of molars (and no premolars). Often when supernumerary dentition occurs in mice, it is within the retromolar space between the molars and incisors (this will be expanded on below). Secondly, there may be differences in expression due to the phylogenetic differences between the parents (also expanded on below). Finally, considering that so few studies focus on dental non-metric traits in hybrids, we do not yet fully understand the mechanisms underlying these traits, which may apply to some taxa (such as primates), but may not apply to mice.

The hybrid mice do, however, appear to express zygomaxillary sutural anomalies, similar to those seen in the above studies. This pattern is not consistent among all mouse hybrids, but it is compelling given that it is seen in the (limited) hybrid research.

HYPOTHESES

I will now evaluate the hypotheses established in Chapter 1 (Introduction) in the context of the results. Each hypothesis will be referred to as “supported” if the results are consistent with expectations established in the hypothesis, or “unsupported”, if not.

HYPOTHESES ESTABLISHED FOR CHAPTER 6 (AS WELL AS INTERSPECIFIC HYBRIDS)

HYPOTHESIS 1: F1 hybrids are larger than a calculated midpoint of parents in measures of cranial and mandibular form relative to the parents: SUPPORTED

The majority of measures were larger than parental midpoint in F1 hybrids (a quantitative genetic definition of heterosis). Many of these measures were consistent in all three intra-specific hybrids studied, and many of these were not only larger than parental average, but larger than parental measures. Furthermore, these patterns appear to be consistent across the different mouse crosses studied for certain measures: facial length and neurocranial height, and mandibular and maxillary alveolar length.

HYPOTHESIS 2: The degree of heterosis (as defined above) seen in F1 hybrids relative to their parent taxa is comparable with that seen in other mammalian hybrids: UNSUPPORTED

There appears to be large disparity in terms of degree of heterosis among hybrids of different taxa. While there was some consistency among the intra-specific mouse hybrids used within this study, the magnitude of heterosis is more extreme that what seen in baboons and tamarins. Furthermore, intra-specific mouse hybrids were more extreme in patterns of heterosis than the interspecific hybrids.

HYPOTHESIS 3: F1 hybrids exhibit intermediate shape relative to their parent taxa: SOMEWHAT SUPPORTED

Generalized Procrustes Analysis eliminated absolute size from the dataset, leaving variables which are interpreted as "shape". When size is removed in this manner, and coordinates used in a Principal Components Analysis, all three crosses exhibited intermediate position along the PC1 axes, which explained between 36-44% of the cranial shape, and 42-47% of the mandibular shape. It is important to note, however, that the shape data are also extreme in some measures, with PC2 of crania and mandibles often displaying extreme hybrid shape. This means that while the bulk of shape variation is intermediate, some aspects of shape variation are more transgressive.

HYPOTHESES ESTABLISHED FOR CHAPTER 7

HYPOTHESIS 1: Like F1 hybrids, multigenerational recombinants are larger than parents: SUPPORTED

Cranial and mandibular size is larger in the B1s and F2s than in parents. It is important to note that measures in these recombinants are not as large as observed in the F1s.

HYPOTHESIS 2: We can use size variables to test for hybridization within a mixed sample. There is an increase in absolute size and size variation of a sample if hybrids are included in that sample, as opposed to a sample with only parents: SOMEWHAT SUPPORTED

There is an increase in average size (and to an extent, variance) in both the cranium and mandible of the intra-specific samples. However, this trend is far clearer in the cranium than in the mandible, the latter of which displayed large confidence intervals.

HYPOTHESIS 3: Multigenerational recombinants are intermediate in shape relative to parents: SOMEWHAT SUPPORTED

All hybrids (F1s, F2s and B1s) were intermediate to the parents, with B1s more similar to the backcrossed parents and F2s overlapping with the F1s. However, like seen in F1s, they are more extreme in some shape variables to the parents, often overlapping with F1s.

HYPOTHESIS 4: F2 and B1 generations are more variable in shape than parents or F1 hybrids: SUPPORTED

F2s and B1s exhibit greater shape variation in the cranium and mandible, although this pattern is particularly pronounced in the cranium. F2s are also more variable than B1s.

HYPOTHESIS 5: F2 hybrids overlap in shape with the F1 hybrids, and B1s are intermediate in shape, between hybrids and parents: SUPPORTED

The F2s overlapped greatly with the F1s in shape space, and the B1s were intermediate between the F1s and the parents with which they were backcrossed. There is, however, greater shape variation in the F2s and B1s than in the F1s.

HYPOTHESIS 6: There is a breakdown in integration and covariation of the cranium and mandible in subsequent multigenerational recombinants compared with parents: SOMEWHAT SUPPORTED

Modularity of cranial elements seemed just as likely in parents and hybrids, and appeared to be hereditary. Covariance within these modules, however, was more complex. In the mandible, especially, covariance matrices of backcrosses were more poorly correlated with the parents and other hybrids.

HYPOTHESES ESTABLISHED FOR CHAPTER 8

HYPOTHESIS 1: Atypical non-metric trait variation occurs at a higher frequency in hybrids and multigenerational recombinants, relative to parents: SOMEWHAT SUPPORTED

Unlike that seen in baboons, the mice do not appear to have high proportions of dental anomalies relative to purebreds/parents. While there are some traits which occur more frequently in hybrids than in parents (e.g. extra zygomatic sutures), the results are not as clear and strong in the mice.

HYPOTHESIS 2: Atypical non-metric traits are more likely to occur bilaterally in hybrids, relative to parents: UNSUPPORTED

None of the features expressed in significantly higher proportions in mouse hybrids relative to parents were bilateral.

UNDERLYING CAUSES OF HYBRID MORPHOLOGIES

THE POTENTIAL EFFECT OF PHYLOGENETIC DIVERGENCE ON SIZE AND TRAIT ANOMALIES

As highlighted in Chapter 3, Stelkens and colleagues used transgression estimates from the body size of cichlid hybrids to determine whether transgression (in body size) was associated with genetic and phenotypic distance (Stelkens, Schmid *et al*. 2009). They also looked at transgressive traits (behavioural, morphological and physiological) within the literature of both plant and animal hybrids to determine potential correlations of transgression with genetic distance (Stelkens, Seehausen 2009). Within their analyses, transgression was more tightly correlated with genetic distance than phenotypic distance. This was particularly strong in F2 hybrids, where genetic distance appeared significantly

linearly correlated with transgression. These studies supported the hypothesis that transgression is caused primarily by differences in complementary gene action or epistasis, where the greater the genetic distance, the more likely mutations will accumulate, adapting the genomes in differing ways, and, thus, the more likely transgression will occur on re-merger of these differentiated genomes.

There are, however, a few limitations to the current model in its applicability to the hominin fossil record. Transgression in behavioural traits, or morphological traits which affect soft tissue, is important for understanding hybridization in living organisms, but is difficult to extrapolate or visualise in the past. It is therefore essential to look at transgression of traits which may preserve, and, in the context of this thesis, traits visible on the skeleton. However, this research has only been conducted on a few hybridizing taxa.

Another limitation in building a model is the difficulty of comparing divergence among mammalian taxa with different generational lengths (average age of parents at the birth of offspring), population sizes, mating strategies and mutation rates. Substitution rates in New World Monkeys, for instance, are higher than in apes, and higher in chimps and gorillas than in humans (Moorjani, Amorim *et al*. 2016), making comparing phylogenetic distances among groups difficult. Comparing these with extinct taxa complicates this further. For comparing hybridizing taxa with different estimated generational times, an average number of generations since initial divergence is calculated. We can also make some assumptions about generational time of fossil taxa by extrapolating modern analogues into the past, or by using dental techniques to estimate growth rates of taxa in the fossil record. These estimates, and genetic distances (from the literature) among hybridizing taxa mentioned in this chapter, are recorded in Table 9.1.

TABLE 9.1. COMPARING DIVERGENCE AMONG DIFFERENT HYBRIDIZING GROUPS. DIVERGENCE ESTIMATES FROM THE LITERATURE (LANGERGRABER, PRUFER *ET AL*. 2012, GUÉNET, BONHOMME 2003), ESTIMATED NUMBER OF GENERATIONS CALCULATED FROM THE LITERATURE BASED ON ESTIMATED DIVERGENCE AND GENERATION LENGTH (LANGERGRABER, PRUFER *ET AL*. 2012, PACIFICI, SANTINI *ET AL*. 2013), AND GENETIC DISTANCES CALCULATED FROM MTDNA IN THE LITERATURE (NEWMAN, JOLLY *ET AL*. 2004, CROPP, LARSON *ET AL*. 1999, SHE, BONHOMME *ET AL*. 1990, CASTRESANA 2001) ARE ALL RECORDED. CRANIAL HETEROSIS AND NON-METRIC TRAITS ARE BASED ON THIS THESIS AND SEVERAL OTHER STUDIES LOOKING AT MEASUREMENT ESTIMATES IN THE LITERATURE (ACKERMANN, ROGERS *ET AL*. 2006, DE KLERK 2008, CHEVERUD, JACOBS *ET AL*. 1993, ACKERMANN, SCHROEDER *ET AL*. 2014, ACKERMANN, BRINK *ET AL*. 2010).

	Cranial heterosis	Non-metric anomalies	Genetic distance	Estimated initial divergence	Est. number of generations
Olive-Yellow baboons	Low	High	0.005	~350 000 years	~35 000
Saddle-back tamarins	Medium	—	0.046	~3 000 000 years	~352 900
Black and blue wildebeest	Medium	High	0.43	~2 000 000 years	~300 000
Mus musculus subspp.	High	Medium	0.82	~450 000 years	~265 000
Mus spp.	Medium	Low	1.13	~1 500 000 years	~880 000
Modern humans-Neanderthals	—	—	0.02	~650 000 years	~23 300
Human-chimp	—	—	0.155	~6 000 000 years	~377 360

Table 9.1 shows divergence estimates in terms of average time since divergence, number of generations and genetic distance (from mtDNA sequences, particularly cytochrome b, recorded within the literature) among hybridizing (or potentially hybridizing) taxa. It also summarises the extent to which non-metric anomalies and cranial heterosis affects resultant recombinants. Although the number of studies from which to draw this kind of information is limited, it may be possible to use this to build a foundation for understanding the effect of divergence of parents on hybrid skeletal morphologies. It appears as though closely related parent populations will produce recombinants with average morphology that is near the average of the two parent taxa, but potentially have a high degree of non-metric anomalies.

Within chapters 6 and 7, intra-specific hybrids were shown to be larger than parental average in many traits, and often were larger than parents. However, a comparable level of size transgression was not seen in inter-specific mouse hybrids, and typically not observed to the same extent in baboons and tamarins (Cheverud, Jacobs *et al*. 1993, Ackermann, Rogers *et al*. 2006). This may be due to several factors, listed earlier in the discussion, but one reason that appears to be supported by these results is that the effect of phylogenetic distance among the parents, may be complex: increasing size with increasing phylogenetic distance up until a point, then decreasing again. This creates a dome shape, or negative parabolic shape relationship between phylogenetic distance and size transgression.

This is supported by research conducted by Rieseberg and colleagues, who have shown that heterosis is extreme in hybrids between domesticates relative to those between wild populations, and in the hybrids of more closely-related parents, than those of divergent parents (Rieseberg, Archer *et al*. 1999). There therefore appears to be a relationship between transgressive/heterotic phenotypic effects and phylogenetic distance, although this is likely further affected by genetic drift and inbreeding. Furthermore, although non-metric skeletal traits in hybrids are currently studied in only a few groups, it is possible that expression of these traits is also affected by divergence. Although data is currently limited, it is possible that unusual non-metric trait expression occurs in higher frequency in hybrids with only minimally-diverged parents, decreasing with increased phylogenetic divergence. Both these features ultimately reduce as parents diverge further.

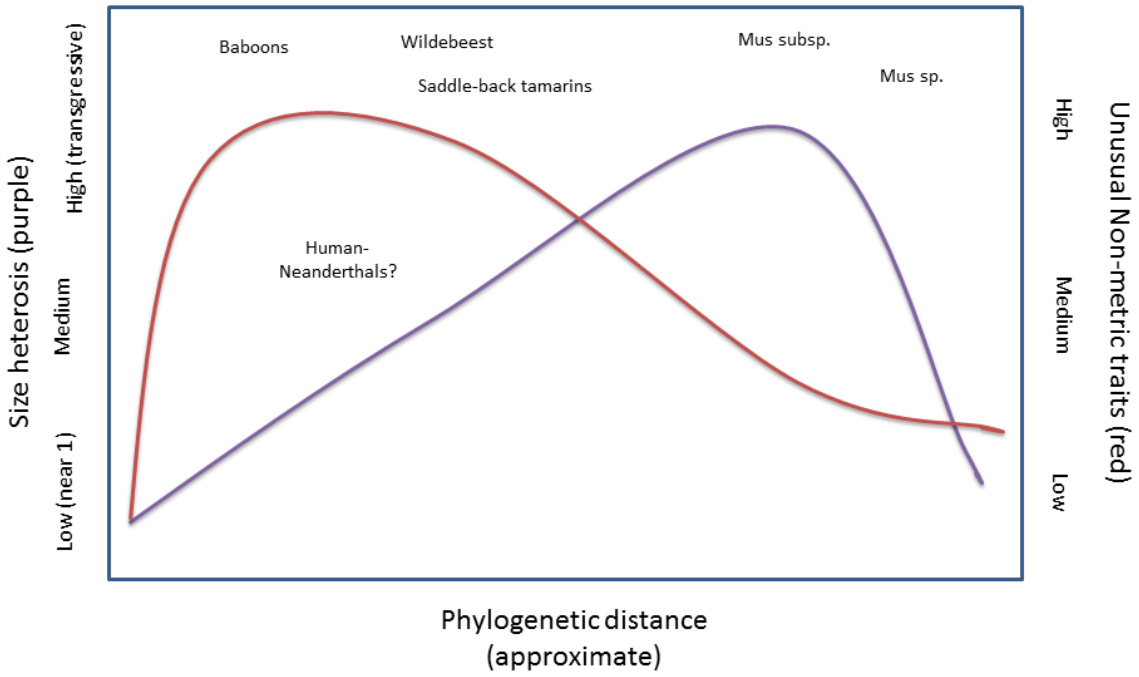


FIGURE 9.1. PROPOSED NON-METRIC AND HETEROTIC TRENDS IN HYBRIDS AND RECOMBINANTS, WITH INCREASING PHYLOGENTIC DISTANCE AMONG PARENTS.

It appears as though as parent populations become more divergent and mutations increase, the hybrids are more likely to exhibit heterotic or transgressive measures on average (summarised in Figure 9.1.). This may be exacerbated by inbreeding, where alleles are more likely to be fixed within a population. However, as divergence increases further, the measures once again reduce in terms of

size transgression. It is important to note that this does not appear predictive of speciation, with some taxa showing signs of speciation (such as post- or pre-zygotic isolation) with very little divergence.

CRANIAL DEVELOPMENT AND HYBRIDIZATION

Mouse cranial and mandibular variation has been studied by a number of researchers over the past few decades, in order to better understand the genetic and developmental underpinnings of the morphologies of these structures (Pallares Amaya 2015, Pallares, Turner *et al*. 2016a, Pallares, Carbonetto *et al*. 2015, Percival, Liberton *et al*. 2016b, Mikula, Auffray *et al*. 2010, Mikula, Macholán 2008, Alibert, Fel-Clair *et al*. 1997, Auffray, Alibert *et al*. 1996b, Debat, Alibert *et al*. 2000, Renaud, Alibert *et al*. 2009, Renaud, Alibert *et al*. 2012, Baird, Macholán 2012, Macholán 1996, Willmore, Roseman *et al*. 2009, Klingenberg, Navarro 2012). The mandible of house mice, in particular, has been used extensively to model complex morphological structures (Klingenberg, Navarro 2012, Atchley, Hall 1991, Sage, Atchley *et al*. 1993). The mandible is formed from embryonic cells from both the cranial neural crest and from the paraxial mesoderm making it an ideal simplified model for the head (Chai *et al* 2000, Depew *et al* 2002). Furthermore, continuous craniofacial and mandibular morphology across the natural hybrid zone between *M. m. musculus* and *M. m. domesticus*, discussed earlier in this chapter, supports a scenario of a polygenic basis for these structures (Pallares, Turner *et al*. 2016a, Mikula, Auffray *et al*. 2010), best explaining the intermediate shape morphologies seen in the cranium and mandible of the hybrids.

When it comes to larger-than-parental average size in the hybrid cranium and mandible, a number of underlying developmental and genetic reasons may be implicated. The rate and timing of hormone and transcription factor release will affect element size in all stages of development: the growth and proliferation of progenitor cells; the effects of regulatory cells (in the bone, this is often osteoclasts and osteoblasts) throughout early development and into adulthood; rate and duration of growth periods during childhood; and secondary (adult) growth (Lieberman 2011, Atchley, Hall 1991). Any influence on hormone release (timing, duration and concentration) in any one or combination of stages, may lead to heterotic or transgressive size in hybrids. This could influence the cranium and mandible as a whole, but these effects may also be localised: for instance during facial growth, possibly explaining longer facial lengths, or development of the tooth row, effecting alveolar length (Ackermann 2007).

Extended facial growth duration may be implicated in dental morphogenesis, explaining supernumerary dentition in the hybrids of other animals such as ground squirrels and baboons

(Ackermann 2007, Ackermann, Rogers *et al*. 2006, Ackermann, Schroeder *et al*. 2014, Goodwin 1998). This was not seen in mouse hybrids. However, it is important to note that dentition in mice is highly reduced compared with other mammals: one incisor and three molars per quadrant, separated by a toothless diastema. This suggests that it might not be reasonable to expect expressions of dental traits comparable to what was seen in the hybrid baboons and other mammals. However, there is some evidence from spliced mice with ectodysplasin (involved in ectodysplasin-Edar signalling) overexpression for extra molars in the mandible (in front of LM1), which occur, frequently bilaterally, and often resemble premolars in other mammals (Mustonen, Pispa *et al*. 2003, Ackermann 2007). Similarly, RunX2, despite being essential for dental morphogenesis, is also important for inhibiting extra dental formation, particularly in the mandible (Aberg, Cavender *et al*. 2004).

The hybrids bred in this study do appear to resemble other mammalian hybrids (wildebeest and baboons) in the relatively high frequency of zygomatic sutures. Sutures of the zygomatic bones and maxillary bones (both derived from the first pharangeal arch from the mesoderm) have been remarked upon in the yellow-olive baboon hybrids (Ackermann, Rogers *et al*. 2006, Ackermann, Schroeder *et al*. 2014). In humans, the pharangeal arches, which give rise to these structures, are seen in the fourth week of development, implying that these morphologies are affected early on in development (Vrana, Fossella *et al*. 2000). Aberrant hybrid morphologies (large size, unusual morphologies) may be explained by epigenetic effects: a breakdown in chromatin integrity, a skewing of X-chromosome inactivation and the effects of imprinting (Vrana, Fossella *et al*. 2000, Wolf, Brodie III *et al*. 1998, Wolf, Oakey *et al*. 2014, Michalak 2009).

In plants, massive regulatory change, inherent in the combining of genomes, is referred to as "genomic shock" (Comai, Madlung *et al*. 2003). Such effects may be seen in animals as well, possibly affecting post-zygotic fertility and ultimately leading to infertility (Vrana, Fossella *et al*. 2000, Wolf, Brodie III *et al*. 1998, Michalak 2009). Perturbed expression of growth-related IGF2 and other growth-related genes (such as *Peg3*, often responsible for overgrowth) have been implicated in certain *Peromyscus* hybrids (field mice; Duselis, Vrana 2010). These effects, spread over multiple genes, compound to form large-scale morphological changes (Vrana, Fossella *et al*. 2000). Such explanations may be better suited for explaining patterns seen in hybrids among multiple different species than, say, Dobzhansky-Muller incompatibilities, which implicate isolated or specific genes. That's not to say that such incompatibilities do not affect individual crosses, but that commonalities and trends seen in multiple hybrids across many taxa, may be better explained by divergence affecting developmental coordination.

SHIFTING TENSION ZONE IN THE MIDDLE TO LATE PLEISTOCENE

Research on wild-derived mouse hybrids and morphologies in the mouse tension zone in Europe (Pallares, Turner *et al*. 2016a), may not be useful for identifying individual hybrid specimens. However, it could be used to explain trends seen in the hominin fossil record. Tension zones, such as seen in these mice in Europe, may allow for good models for what we see in late Middle Pleistocene hominins in the Levant. In these hominins, some individuals, such as the Skhūl specimens, more closely resemble modern humans, and others, such as Amud, more closely resemble Neanderthals. However, many specimens in West Asia in the Middle Pleistocene appear to exhibit mixed archaic morphologies, despite them being categorised as modern human or Neanderthal (Trinkaus 2007, Trinkaus, Moldovan *et al*. 2003). Changes over time in this region may not reflect a "replacement" of Neanderthals and humans, but merely a geographic shift of the tension zone, reflected in the cranial morphologies. In this scenario, we would not be looking for groups of individuals which have a large degree of morphological variation. Instead, individuals which are geographically and temporally proximate will have a similar proportion of "human-like" or "Neanderthal-like" morphologies, reflective of their position within the tension zone.

Multiple scenarios could have influenced the movement of the tension zone: environmental changes, social and technological advancement from either group, or, indeed, any one or combination of factors which have formally been proposed as reasons for "replacement". It is also likely that by the Late Pleistocene, beneficial haplotypes involved in immunity or survival within Eurasia could have stabilized within populations with greater recent-African ancestry. Such a scenario would have allowed for modern human expansion by nullifying some of the biological factors stabilizing the tension zone.

It is currently not well understood as to how long some of the hybrid features seen in this thesis and other studies (unusual non-metric traits and cranio-mandibular heterosis) may persist in a population after the cessation of contact. There is currently no support for an increase in cranio-mandibular size towards the centre of the European mouse hybrid zone, despite genetic evidence for high levels of recombination (Pallares, Turner *et al*. 2016b). This implies that significantly larger size in recent recombinants, as discovered in laboratory experiments, may not retained at significant levels in further recombinants. However, in the study on gorillas, it was estimated that unusual trait morphologies and larger-than-intermediate cranio-facial measures may persist 80 Ka after secondary

contact (Ackermann, Bishop 2010), but other research has brought hybrid origins of these gorillas into question (Tocheri, Dommain *et al*. 2016). Experimentation on hybrid sunflowers has also supported the persistence of unusual hybrid phenotypes into further generations (Lexer *et al.* 2003, Rieseberg *et al.* 2003).

It is possible that cranio-mandibular non-metric trait variation across the European mouse hybrid zone may better reflect the breakdown in genetic coordination brought about through hybridization, yet is not currently well-studied. Features such as supernumerary teeth have been recorded in ground squirrels and baboons within hybrid zones (Ackermann, Schroeder *et al*. 2014). External morphologies, such as pelage pattern, or genitalia colour and morphology, have been noted in primate tension zones (or hybrid zones more broadly). These features do not preserve, but hint at similar developmental changes. More research needs to be made on unusual trait morphologies within hybrid zones in order to better understand their persistence.

HOMININ MORPHOLOGIES CONSISTENT WITH HYBRIDIZATION

The model elaborated on above may explain hybridization in the late-Middle and early-Late Pleistocene in Europe and Asia. It is likely that during modern human expansion, populations were more representative of combinations of parents and recent reticulates: resembling Neanderthals, humans, and possibly exhibiting hybrid-like traits such as transgressive size or rare non-metric traits.

This is supported by studies on ancient DNA of the Oase I mandible, which has indicated Neanderthal ancestry 6-8 generations prior (Fu, Hajdinjak *et al*. 2015). The morphology is largely AMH-like (reflecting larger proportions of modern human ancestry), yet researchers have identified traits which do not only fall intermediate to Neanderthals and humans, but are instead transgressive (Ackermann 2010, Trinkaus, Moldovan *et al*. 2003). Principal components analyses from one study (Trinkaus, Moldovan *et al*. 2003), show that the Oase I mandible falls well within the range of modern human mandibular measures in the first two principal components, yet is outside of the range of variation for both modern humans (early and Upper Palaeolithic) and Neanderthals in some measures. It was further remarked that the mandibular ramus of this specimen was “exceptionally wide” (Trinkaus, Moldovan *et al*. 2003). Moreover, in molar crown measures, Oase I is extreme to both humans and Neanderthals in some measures and more Neanderthal-like in others. The pattern of progressively larger molars from M1-M3 is very rare in modern humans, yet present in the Oase mandible.

Extraction and analysis of ancient DNA of Oase 2 cranium (associated with Oase I, but of a different individual) has not been published (to date). However, similar discordances in the morphology have

allowed some researchers to suggest recent hybrid ancestry for this specimen as well (Ackermann 2010, Rougier, Milota *et al*. 2007). Rougier and colleagues compared Oase 2 to Upper and Middle Palaeolithic hominins and Neanderthals (Rougier, Milota *et al*. 2007). In many measurements of the cranium, Oase 2 fit comfortably within the range of variation of Upper Palaeolithic modern humans. Yet a few features were more similar to Neanderthals (such as Nasion-Bregma Arch), and measures of the maxillary molar areas were transgressive (larger) than in Middle and Upper Palaeolithic modern humans and Neanderthals.

We can see similar patterns in some other Late Pleistocene hominins that have been proposed as hybrids. For instance, Nazlet Khater, a modern human from Egypt, dated to around 33 Ka (Vermeersch, Gijselings *et al*. 1984), exhibits some archaic features, resembling Oase I in having an unusually broad mandibular ramus, more extreme than late other Pleistocene humans, but also exceeding that of Neanderthals (Trinkaus 2007). This can be compared with the Muierii 1 mandible, which has an exceptionally narrow mandibular ramus. The amount of morphological variation within the Late Pleistocene is also consistent with hybridization.

Mosaic traits and more intermediate morphologies have also been suggested as indicating recent hybrid ancestry of Lagar Velho child and specimens from Mladeč (Duarte, Mauricio *et al*. 1999). Lagar Velho is a particularly interesting specimen, since it is dated a few thousand years after humans are meant to have replaced Neanderthals. However, there are a few traits (mainly postcranial) that do not appear modern. Furthermore, molar dental measurements fall outside the range of Upper Palaeolithic modern humans, and more closely resemble Neanderthals (Duarte, Mauricio *et al*. 1999). Such a mosaic of features, particularly the larger molar size, is consistent with the model proposed here, based on the mouse hybrids.

In both mice and baboons, hybrids tend to differ from parents in the occipital region. It is possibly also worth mentioning that occipital bunning (usually seen as a Neanderthal trait), is present in all pre-30 Ka modern humans in Europe (Smith 2013). However, the extent to which these traits can be connected is unclear.

It is also worth mentioning other potential recombinants. The Krapina hominins have also been shown to have morphologies consistent with hybridization (Ackermann 2010). Many of the individuals are likely to be Neanderthals, exhibiting mainly classic Neanderthal traits. However, a few individuals have more modern appearance (Schwartz, Tattersall 2002), and four individuals have rotated premolars (36% of the sample; Ackermann 2010, Rougier, Crevecoeur *et al*. 2006). Premolar rotation was also noted in high proportions in hybrid wildebeest (Ackermann, Brink *et al*. 2010). There are also

some measurements which are outside of Neanderthal variation, such as supraorbital midpoint projection and thickness (Ahern 2006). Krapina hominins are also more variable in some measurements than other Neanderthals, such as in postglenoid process projection, and are smaller in measures of the mastoid process (Martinez, Quam *et al*. 2006).

The Krapina hominins are dated to 130 Ka, before the time period we currently consider human-Neanderthal hybridization to have taken place. However, (as mentioned in chapter 4) it is currently understood that hybridization events between the two lineages may have taken place further back in time: among early modern humans and Neanderthals 100 Ka and possibly earlier (Kuhlwilm, Gronau *et al*. 2016). Furthermore, we also know hybridization among Neanderthals and Denisovans has also taken place; it is possible that hybridization events potentially influencing Krapina morphology may not be from modern humans, but other Eurasian archaics.

Limitations and Future Research

Limitations

There are clear limitations within this study. Firstly, the morphological link between mouse and hominin hybrids is quite distant. It is essential that more research is conducted on primate hybrid morphologies in order to support or reject the models proposed here. Another limitation is the inbred nature of wild-derived strains. Inbreeding has been shown to influence body and cranial size in captured mice (White 1972, Lynch 1977). Inbreeding depression in the parents, coupled with outbreeding, may lead to morphology in their offspring than can be incorrectly interpreted as transgressive. While we acknowledge that inbreeding plays a significant role in the patterns of hybrid cranial size, this trend is seen in mammalian hybrids (particularly F1 hybrids) both captured and in the wild, albeit not to the same extent. It is also important to note that genetic drift and inbreeding has affected hominins in the past, and similar effects of outbreeding may have influenced hominin morphologies. Outbreeding alone, however, does not explain the relatively low level of heterosis seen in the interspecific hybrids.

Future Research

This thesis is a smaller part of a larger, ongoing Mouse Hybrid Project, briefly introduced in Chapter 1. As part of this project, several other crosses have been bred to expand our understanding of the effects of hybridization in multigenerational recombinants. These include B1 crosses backcrossed with the other parent, and B2 lineages, where B1s are crossed with parents and F1s, where possible. This should allow us to more fully expand on our understanding of hybrid morphologies, and to explore concepts such as maternal effect and a breakdown of developmental coordination on recombinants. While not a part of this thesis, this research is currently under way, and will more fully inform, or support, the hypotheses explored here.

A second avenue of exploration, currently under way, is in other inter-specific hybrids. Within this dissertation, only 36 WSBxSPRET F1 hybrids were analysed, yet the breeding of these hybrids is continuing. Furthermore, CASxSPRET hybrids are also being bred for comparative analyses. By expanding our inter-specific database, it will be possible to more fully explore the concept of the relationship between phylogenetic distance and hybrid morphology. Another avenue for future research is to evaluate the morphology of *M. m. molossinus*, a sub-specific mouse taxon with hybrid origins (Yonekawa, Moriwaki *et al*. 1988, Yonekawa, Sato *et al*. 2012). By comparing this taxon with its proposed hybrid progenitors (CASTxMUS F1s, and the parents), it may be possible to better understand the extent to which parent and hybrid traits may be retained long after hybridization takes place. A third avenue of analysis lies in analysing fluctuating asymmetry of crania and mandibles in mice and their hybrids.

Within this study, we have used a qualitative measure for sutural complexity. However, in other studies, researchers have used more quantitative approaches to assessing complexity. The use of a qualitative analysis has already been justified, but it is still important to find a more compatible intermediate measure, which can better distinguish between numerous subtle “waves” and larger, singular deviations. Such a measure may help us understand the diversity of parietal sutures among the parents and within the hybrids to better understand the link between sutural formation and inheritance.

One purpose of this thesis is to better integrate our understanding of mouse hybrid morphologies with that of known primate hybrids and proposed hybrid hominin specimens. Here, mouse data were examined in the light of research conducted on baboon and tamarin hybrids, but it is clear more work needs to be done. While comparing the mice with fruit flies, cichlids and ungulates is useful to cement a model, the connection between animal hybrid morphologies and that of hominins lies with a better understanding of primate hybrid phenotypes. Primate hybrid external morphologies are far better

understood (see Chapter 3). One current study underway within the Mouse Hybrid Project, is attempting to figure out a potential connection between pelage and skeletal variation. However, it is still important to expand on our knowledge of hybrid skeletal (particularly cranial) morphologies in primates, if we are to more successfully compare these models with hominin specimens.

In summary, it is important to note that this research is still in its infancy. The Mouse Hybrid Project will continue to add to our understanding of hybrid morphologies by studying other inter-specific mouse hybrids and by continuing the breeding of mice to include more recombinants. It is also important to compare with mouse morphologies in natural mouse hybrid zones. The tension zone in Europe is well studied, but studies on non-metric trait variation in this zone are limited. Additionally, it is important to understand the morphological effects of hybridization in more fit mouse hybrids, such as *M. m. molossinus* in East Asia. Furthermore, far more research needs to be conducted on baboon and other primate hybrid skeletal morphologies, both in terms of craniometric comparisons as well as looking at non-metric cranial traits. Finally, more research needs to be conducted on the post-cranium, especially considering the important differences among humans and Neanderthals lay mainly in post-cranial morphologies.

CONCLUSION

A range of techniques were used to assess cranio-mandibular form and non-metric traits of three intra-specific, and one inter-specific, mouse hybrids. While primates are more appropriate models for hominin evolution and hybridization, research on their morphology is limited by sample availability and ethical considerations. This thesis contributes to a mammalian model by confirming or establishing patterns seen in other research on hybrids (e.g. wildebeest, baboons, tamarins). Here, it is demonstrated that mouse intra-specific hybrids exhibit larger-than-parental cranio-mandibular size, whereas inter-specific hybrids are larger-than-parental-midpoint, but not larger than the larger parent. It is shown that some cranio-mandibular measures appear larger than parental average, or larger than parental, in all hybrids: particularly in the occipital and maxillary/mandibular alveolar regions. It is demonstrated that intra-specific hybrids may exhibit traits such as extra zygomatic sutures and parted frontal bones, but these are not expressed within all mouse hybrid groups. Furthermore, it is demonstrated that a breakdown in integration of structures such as the mandible may explain unique morphologies in recombinants.

So what did a human-Neanderthal hybrid look like? Was Durc, the figurative hybrid in *Clan of the Cavebear*, a mixture of his human mother and Neanderthal father? The data assessed in this thesis shows that hybrids are frequently intermediate, in terms of cranio-mandibular shape, to the parents. It is possible that Durc had morphological anomalies absent or seen in relatively low frequencies in the parent taxa, though the pattern and degree of expression could differ from other organisms, and the genetic distance may not be far enough diverged. Furthermore, it is also likely that some of the cranio-mandibular measures are larger than an expected intermediate value, with some occipital or alveolar measurements being particularly large or even transgressive relative to both parents. It is also possible that the phylogenetic distance between humans and Neanderthals were too small for these traits to be transgressive. The patterns seen in this thesis and in the current literature provide important foundational work, and it is therefore possible to make several broad proposals.

Firstly, mammalian skeletal hybrid morphologies are, like that seen in plants and cichlids, affected by phylogenetic distance. Furthermore, like trends seen in cichlid fish, mammalian hybrids become increasingly transgressive in size with greater phylogenetic distances. However, unlike the trend seen in cichlids, both non-metric traits and large size reduces when hybrids occur among very greatly-divergent taxa (such as seen in the inter-specific mouse hybrids). It is important to note that, regardless of phylogenetic distance, certain parts of the cranium appear to be affected by hybridization, with occipital and maxillary/mandibular alveolar measures appearing relatively larger in hybrids studied here and in the literature.

Secondly, these morphological patterns appear consistent with what is seen in certain known or suggested hominin hybrids. The Oase mandible, a known recombinant, exhibits molars larger than that seen in both modern humans and Neanderthals. This is consistent with what is seen in mice, baboons and tamarin hybrids. Furthermore, the cranium associated with the mandible (but not of the same individual) also exhibits extremely large molars, supporting the hypothesis that this population had multiple recombinants. This supports a scenario of hybridization between humans and Neanderthals in Europe the late Pleistocene.

The hominin fossil record yields highly variable, diversifying taxa, all prone to adaptive and non-adaptive forces of evolution which may, in turn, contribute to the evolution of our lineage. Hybridization, as a first step for allowing gene flow, is a principal force for variation and was likely a crucial part of hominin diversification. While hybridization between Late Pleistocene hominins is best known thanks to scientific advances in ancient DNA analyses, morphology is providing crucial evidence for detecting fossil hybrids: an important contribution for identifying its occurrence during the emergence of our species, but also ultimately among hominin taxa even deeper in the past.

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APPENDICES

APPENDIX 1: R CODE FOR BASIC FUNCTIONS FOR PROCRUSTES VARIANCE, RESAMPLING AND CLASSIFICATION TECHNIQUES (CHAPTER 7)

```
#PROCRUSTES DISTANCES


#Procrustes Distance from mean shape: Function Proc.dist calculates and returns list of Procrustes distances between each individuals and mean shape.
    Proc.dist <- function  (PC) {      # PC is array of aligned Procrusted coordinates (i.e. after GPA).
      p <- dim (PC)[3]
      ms <- apply (PC, c(1, 2), mean)
      dists <- vector (p)
      for(i in 1:p) {
        dists[i] <- sqrt (sum ((PC [,,i] - ms)^2))
      }
      dists
    }
```

```
# CLASSIFICATION

   # using Geomorph package
   # convert to coords for each group
   coordsHybrid <- arrayspecs( )  #array deats, continue for all groups
   coordsParent <- arrayspecs( )
   msParent <- apply (coordsParent, c(1, 2), mean)  #work out mean shape of parent strain

   p <- dim (coordsHybrid)[3]       # save dimensionality of p if unknown
   dists <- vector ("numeric", p)
   for(i in 1:p) {
     dists[i] <- sqrt (sum ((coordsHybrid[,,i] - msParent)^2)) # this is the formula for PD to a mean
   }
   dists
```

```
#Mixed models

   library("dplyr", lib.loc="C:/Program Files/R/R-3.3.0/library")

   #split up tables into parents and F1 hybrids
```

```
  Mean.dist=rep(NA,999) # create table for distribution of means
  Variance.dist=rep(NA,999) # create table for distribution of variances

  for (i in 1:999) {
   sample.parent<- sample(Parent$Centroid.Size, number_of_parents, replace=TRUE) # resampling parents
   sample.F1 <- sample(F1$Centroid.Size, number_of_F1s, replace=TRUE) # resampling F1 hybrids-size= 0 to 30/50
   Sample<- c(sample.parent, sample.F1) # combined numbers
   Mean.dist [i] <- mean(Sample) # mean of combined numbers
   Variance.dist [i] <- var(Sample) #variance of combined numbers
  }
  1_var_low <- quantile(Variance.dist,0.025)
  1_var_high <- quantile(Variance.dist, 0.975)
  1_var <- mean(Variance.dist)
  1_mean_low <- quantile(Mean.dist, 0.025)
  1_mean_high <- quantile(Mean.dist, 0.975)
  1_mean <- mean(Mean.dist)
```

APPENDIX 2: NON-METRIC TRAIT COUNTS (DEFAULT OCCURS BILATERALLY, WHERE RELEVANT; OTHERWISE NUMBERS OF SPECIMENS WHERE FEATURES APPEAR ONLY ON THE LEFT OR RIGHT ARE RECORDED AS L OR R).

	n	Teeth									
		Rotated	Missing M3s	Broken teeth/ bifurcated teeth	Teeth too worn	Peg/ reduced	Cusps M1s<6	Cusps M1s>6	Cusps M2s>4	Cusps M3s<3	Cusps M3s>3
CAS	50	0	7 (1L, 1R)	3	3	2	1 (1L)	0	21 (7L,3R)	2 (1L,1R)	0
WSB	50	0	0	0	1	0	2 (2L)	2-1L,1R	26 (4L,6R)	0	5 (4L)
CZE	50	0	6 (3R,1L)	0	0	0	5 (2L)	1-1B	28 (8R,7L)	0	1 (1R)
SPR	50	2	15 (4R,2L)	0	21	0	0	0	11 (5L,1R)	2 (1B,1L)	9-(2L,1R)
CASxWSB	50	0	0	0	0	0	0	0	43 (8L,1R)	0	0
CASxCZE	50	0	0	0	0	0	0	0	36 (6L)	0	0
WSBxCZE	50	0	0	0	0	0	2 (1L,1R)	0	41 (2L)	0	0
SPRxWSB	36	0	0	0	0	0	0	0	11 (5L)	0	5 (2L)
CASxWSB_F2	50	0	1 (1L)	0	0	1	0	1-1B	32 (3L,5R)	1 (1B)	3 (1L)
CASxCZE_F2	50	0	2 (1R)	0	0	0	1 (1L)	1-1L	35 (8L,2R)	2 (2R)	1 (1L)
(CASxWSB)xCAS	50	0	0	0	0	0	0	0	46 (10L,3R)	1 (1B)	2 (2R)
(CASxCZE)xCZE	48	0	0	0	0	1	0	0	17 (7L,3R)	0	5 (2L,1R)
(WSBxCZE)xWSB	50	2	0	0	5	1	0	0	35 (6L,2R)	2 (2B)	4 (2L,1R)

	n	Suture fusion- present and laterality if applicable				Extra sutures- present and laterality if applicable					
		Nasal fusion	Basi-sphenoid/ basi-occipital	Basi-sphenoid/ pre-sphenoid fusion	Dorsal frontal fusion	Nasal	Maxilla	Zygomatic	Parietal	Interparietal	Occipital
CAS	50	0	1	0	0	0	0	6 (5L,1R)	0	0	47 (5L,5R)
WSB	50	0	2	15	0	0	0	0	2	0	0
CZE	50	0	0	0	0	0	0	0	0	0	0
SPR	50	18	0	0	4	0	0	2 (2L)	0	0	2 (2R)
CASxWSB	50	0	0	0	1	0	0	1 (1L)	0	0	0
CASxCZE	50	0	0	0	0	0	0	0	0	0	1 (1L)
WSBxCZE	50	0	0	1	0	0	0	0	0	1 (1R)	0
SPRxWSB	36	0	0	0	0	0	1	22 (12L)	0	0	0
CASxWSB_F2	50	0	0	0	0	0	0	6 (4L)	0	0	8 (1L,3R)
CASxCZE_F2	50	0	0	0	0	1	0	8 (1L,1R)	0	0	3 (1L,2R)
(CASxWSB)xCAS	50	0	0	0	0	0	0	6 (5L)	0	0	22 (4L,8R)
(CASxCZE)xCZE	48	0	0	0	0	0	0	2 (2L)	1	0	0
(WSBxCZE)xWSB	50	0	0	0	0	0	0	5 (3L, 1R)	0	0	0

	n	Wormion Bones- present and laterality if applicable					Hyperstotic/hypostotic				
		Lamdoidal	Asterion	Coronal	Bregmatic	Pterion	Parietal notch	Bridging on incisive foramen	Parted frontal bones-conservative	Parted frontal bones-not conservative	Frontal fontanelle
CAS	50	0	0	0	0	0	0	0	0	2	0
WSB	50	0	24 (4L,13R)	0	0	0	1	0	0	1	0
CZE	50	0	0	0	0	0	0	0	2	5	0
SPR	50	0	27 (7L,10R)	0	0	0	0	0	0	3	0
CASxWSB	50	0	1 (1L)	0	0	0	0	0	14	24	1
CASxCZE	50	0	1 (1L)	0	0	0	0	0	1	5	0
WSBxCZE	50	0	1 (1R)	0	0	0	0	0	5	9	0
SPRxWSB	36	0	5 (1L,3R)	0	0	0	2 (2R)	0	0	2	0
CASxWSB_F2	50	0	2 (1L,1R)	0	0	0	0	1	2	4	0
CASxCZE_F2	50	0	0	0	0	3 (1L)	0	2	1	3	0
(CASxWSB)xCAS	50	1	1 (1R)	0	0	5 (2L,1R)	1B	1	8	11	1
(CASxCZE)xCZE	48	0	0	1 (1R)	1	0	2 (1L,1R)	0	1	2	0
(WSBxCZE)xWSB	50	0	16 (7L,8R)	0	0	0	2 (2R)	0	4	15	0

	n	Foramina					
		Frontal>1	Frontal<1	maxillary foramen I<1	maxillary foramen I>1	Maxillary foramen II>1 (uni)	Maxillary foramen II<1 (uni)
CAS	50	11-5 (1R,2Ls), 1 (1R,3Ls), 4 (2R,1L)	3-2 (1L only), 1(1R only)	28-8 (1L only),4 (R only)	10-1 (1R,4L),1 (1R,2L),3 (2R,1L)	0	50-3 (1L),5 (1R)
WSB	50	7-2 (1R,2Ls),3 (2R,1L), 1 (3R,2L)	0	28-7 (1L only),13(R only)	2-1 (2R,1L)	0	44-5(1L),11(1R),1(2L),4(2R)
CZE	50	4-1 (1R,2Ls)	7-2 (1L only), 5 (1R only)	50-4 (1L only),3(R only)	0	14-3(1R,2L), 1(1R,3L),6 (2R,1L),1 (3R,1L)	7-3(1L),1(1R),1(2L),1(2R)
SPR	50	1-1 (1R,2Ls)	1-1 (1L only)	44-10 (1L only),8(R only)	2-1 (2R,1L)	0	48-2(1L),4(1R)
CASxWSB	50	1-1 (2R,1L)	0	22=8-10 (1L only), 12(R only)	4-1 (1R,2L),1 (2R,1L)	0	46-2(1L),12(1R)
CASxCZE	50	2-1 (2R,1L)	1-1 (1L only)	34-15 (1L only),9(R only)	4-1 (1R,2L),1 (2R,1L)	1-1 (2R,1L)	36-8(1L),7(1R),1 (1R)
WSBxCZE	50	4-2 (1R,2Ls), 2 (2R,1L)	0	41-19 (1L only),6(R only)	0	12-3 (1R,2L),4 (2R,1L)	8-1(1L),2(1R),1 (2L)
SPRxWSB	36	0	8-4 (1L only),4 (1R only)	33-3 (1L only),5(R only)	0	1-1(1R,2L)	23-2(1L),17(1R),1(2R)
CASxWSB_F2	50	2-1 (2R,1L)	1- 1(1R only)	45-13 (1L only),5(R only)	0	0	36-5(1L),10(1R)
CASxCZE_F2	50	7-3 (1R,2Ls), 1(1R,3Ls),1 (2R,1L)	4-2 (1L only),2 (1R only)	48-6 (1L only),6(R only)	1-1 (2R,1L)	5-1(1R,2L),3 (2R,1L)	21-8(1L),5(1R)
(CASxWSB)xCAS	50	3-2 (1R,2Ls), 1 (2R,1L)	2-2 (1L only)	42-7 (1L only),10(R only)	0	1-1 (2R,1L)	45-1(1L),8(1R)
(CASxCZE)xCZE	48	1-1 (1R,2Ls)	14-4 (1L only), 10(1R only)	44-6 (1L only),3(R only)	0	11-3(1R,2L),5 (2R,1L)	10-3(1L),3(1R)
(WSBxCZE)xWSB	50	3-1 (1R,2Ls), 1 (2R,1L)	2-1 (1L only),1 (1R only)	39-9 (1L only),11(R only)	0	15-3(1R,2L),6 (2R,1L),1(2R,3L),1(3R,1L)	24-7(1L),8(1R),2 (2L),1(2R)

	n	Foramina					
		Foramen spenoidal ventral >1	Foramen spenoidal ventral =0	Postcondylar canal<1	Postcondylar canal>1	Preorbital foramen<1 (uni)	Preorbital foramen>1 (uni)
CAS	50	25	7	3-1 (1L), 2 (1R)	30-13(2B), 6(1R,2L),7(2R,1L),1 (2R,3L),2(3R,1L),1(3R,2L)	37-8 (1L),7 (1R), 1 (2L)	2-1 (2L)
WSB	50	9	21	0	50- 21 (2B),2(1R,2L),3(2R,1L),15 (3B),2(2R,3L),6(3R,2L),1(3R,4L)	5-2(1L),3(1R)	3-1(2R,1L)
CZE	50	4	30	1-1 (1L)	41- 12 (2B),6(1R,2L),3(2R,1L), 6 (3B),6(2R,3L),3(3R,1L),6(3R,2L)	24-5(1L),7(1R)	3-1 (3R,2L)
SPR	50	3	40	8-1 (1L),2 (2L), 3(2R)	30- 11(2B),5(1R,2L),4(2R,1L), 3(3B),1(2R,3L),1(3R,1L),2(3R,2L),1 (3R,5L),1 (4R,2L),1 (4R,1L)	37-5(1L),4(1R),1 (2L), 2(2R)	3-1 (2L), 2(2R)
CASxWSB	50	8	27	0	45- 21 (2B),4(1R,2L),4(2R,1L), 5(3B), 3(2R,3L),1 (3R,1L),6(3R,2L),1 (4R,2L)	11-5(1L),1(1R)	1
CASxCZE	50	10	17	4-1 (1L),3(1R)	29- 19 (2B),5 (1R,2L),4(2R,1L),1(3B)	1-1(1R)	4-1(1R,2L)
WSBxCZE	50	10	22	1-1 (2L)	25- 15 (2B),5(1R,2L),4(2R,1L),6(3B),1(4B),4(2R,3L),1 (2R,4L),2 (3R,2L),3(3R,2L),1 (4R,2L)	5-3(1L),1(1R)	5-2 (2R,1L)
SPRxWSB	36	18	19	2-2(1R)	27- 12 (2B),2(1R,2L),5(2R,1L),1(3B),1(4B),3(2R,3L),2(3R,2L),1(4R,2L), 1 (3L),1 (3R)	11-3(1L),6(1R)	8-3(2R,1L)
CASxWSB_F2	50	21	9	4-3(1R)	34- 12 (2B),2(1R,2L),3(2R,1L),7(3B),1(4B),6(2R,3L),1 (2R,4L),2(3R,1L)	15-3(1L),2(1R),1 (2L)	11-1(1R,2L),3(2R,1L),1 (2L)
CASxCZE_F2	50	18	11	14-6 (1L),4(1R),3 (2L),1 (2R)	25- 10 (2B),5(1R,2L),5(2R,1L),1(3B),2(2R,3L),2(3R,2L)	19-8(1L),4(1R),1 (2L),1 (2R)	15-3 (1R,2L),3(2R,1L),1 (2L),1 (2R)
(CASxWSB)xCAS	50	36	0	3-1 (1L),1(1R)	33- 15 (2B), 8(1R,2L),2(2R,1L),2(3B), 1(4B),1(2R,3L),2(3R,1L),2(3R,2L)	12-3(1L),2(1R),2 (2L)	20-3(1R,2L),4(2R,1L),2 (2L)
(CASxCZE)xCZE	48	6	33	6-2 (1L),2 (2L),2 (2R)	26- 10 (2B),5(1R,2L),3(2R,1L),3(3B),2(2R,3L),2(3R,2L),1 (3R),1 (4L)	23-7(1L),5(1R)	11-2(1R,2L),2(2R,1L)
(WSBxCZE)xWSB	50	26	13	4-1 (1L),1(1R),1 (2L),1 (2R)	29- 7 (2B),7(1R,2L),4(2R,1L),6(3B),3(3R,2L),1 (4R,2L),1 (4R,3L)	5-2(1L),3(1R),1 (2R)	25-3(1R,2L),4(2R,1L),1 (2R)

	n	Foramina		Parietal suture				
		Mental foramen>1	Extra sutural incisive foramen>0	1	2	3	4	5- side and near L (lambda), B (bregma), M (middle)
CAS	50	0	45-2(2B),8 (1L),7 (1R),1 (2L), 1 (2R), 1 (2R,1L)	19	20	4	2	5-2LL,3RL
WSB	50	47-8(1R,2L),5(2R,1L)	3-1(1L),1(1R)	2	9	21	11	7-1LL,3RB,2RL,1RM
CZE	50	37-14(1R,2L),9(2R,1L)	30-2(1L),3(1R)	6	29	13	2	0
SPR	50	0	1-1(1R)	0	4	14	32	0
CASxWSB	50	20-8(1R,2L),6(2R,1L)	5-1(1R)	0	16	18	12	4-1LB,1LL,2RL
CASxCZE	50	2-1 (1R,2L),1 (2R,1L)	45-,1(1B),3(1L),3(1R),2 (1R,2L)	15	26	9	0	0
WSBxCZE	50	46-15(1R,2L),11(2R,1L)	24-7(1L),7(1R), 1(2L)	5	16	13	5	11-2LB,2LL,2RB,3RL,2RM
SPRxWSB	36	0	4	3	16	13	4	0
CASxWSB_F2	50	18-5(1R,2L),6(2R,1L)	9-1(1L),2(1R)	8	20	11	6	5-1LL,4RL
CASxCZE_F2	50	7-4(1R,2L),1(2R,1L)	31-8(1L),6(1R),1 (2L)	14	21	12	1	2-1LM,1RL
(CASxWSB)xCAS	50	10-3(1R,2L),6(2R,1L)	18-3(1L),7(1R), 1 (2R)	10	29	8	1	2-2RL
(CASxCZE)xCZE	48	8-5(1R,2L),1(2R,1L)	39-2(1L),5(1R), 1(2R,1L)	18	24	4	1	0
(WSBxCZE)xWSB	50	33-7(1R,2L),16(2R,1L)	12-1(2B),4(1R)	0	12	16	18	4-1LB,1LM,1RL,1RM

GLOSSARY

Below is a list of terms used in this thesis which may not be commonly known.

Archaic This term is used to refer to hominin lineages more divergent from modern humans. It may then include Neanderthals, Denisovans, or even more greatly divergent hominin lineages, which may have interbred with modern humans (in and out of Africa) or with each other.

Allele Variant of a gene found at specific locus.

Dysgenesis Within this thesis, dysgenesis refers to measured features which are smaller or reduced in the hybrids relative to parents and/or relative to the mid-parental value. It is often used to refer to sterility or lowered fitness within hybrids and recombinants.

Gene Flow The spread of particular alleles within a population and between populations resulting from hybridization and subsequent recombination.

Genetic Barrier Loci Genomic regions reinforcing or enhancing reproductive isolation.

Heterosis In this thesis, heterosis is used to describe measured features which are larger in hybrids than the mid-parental value.

Heterozygosity Proportion of loci with different alleles stemming from divergent/ different populations.

Hybrid Offspring of a cross between genetically different or phylogenetically divergent taxa (verb: hybridize).

Hybrid zone Geographic area in which two populations once separated by a geographic barrier hybridize after the barrier has broken down.

Hybridization The formation of a hybrid.

Hybrid sterility Sterility of an individual arising from the fact that it is a hybrid.

Hybrid swarm Population consisting primarily of hybrids and their descendants.

Hybrid vigour When the hybrid is more vigorous or fit that either of the parents, often due to increased heterozygosity.

Introgression The gradual movement of genes from one species to another when there is some hybridization between the two.

Interbreeding The crossing of different taxa.

Late Pleistocene The geochronological age, 126 Ka until the Holocene, 12 Ka; the time period associated with modern human expansion from Africa.

Loci The positions on a chromosome or genetic sequence where genes may be found (singular: locus).

Middle Pleistocene Geochronological age, 781-126 Ka.

Overdominance The condition where a heterozygote phenotype lies outside the range of either of the homozygote phenotypes.

Reticulation Network-like evolution of organisms, through repeated hybridization events, or other forms of horizontal genetic transfer.

Transgressive When features are more extreme in the hybrids than in the parents.

Recombinant Genotypes produced because of genetic recombination.

Recombination Process by which sexually-reproducing populations exchange DNA between homologous chromosomes, producing gametes with alleles from both parents on the same chromosome.